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Subject: FW: sequence search 09/446,681

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-----Original Message-----

From: Vogel, Nancy
Sent: Friday, November 14, 2003 12:10 PM
To: STIC-ILL
Subject: sequence search 09/446,681

Please do a sequence search, including interference search, for nucleotide numbers 295 to 1450 of SEQ ID NO: 1 of 09/446,681 and return paper results to me ASAP

Thanks,
Nancy Vogel
Art Unit 1636
CM12 C01
(703) 308-4548

Searcher: _____
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Location: _____
Date Picked Up: _____
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TYPE OF SEARCH:
NA Sequences: _____
AA Sequences: _____
Structures: _____
Bibliographic: _____
Litigation: _____
Full text: _____
Patent Family: _____
Other: _____

VENDOR/COST (where applic.)
STN: _____
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09/446681
A4#14

1. Document ID: US 20010008770 A1

L3: Entry 1 of 28

File: PGPB

Jul 19, 2001

PGPUB-DOCUMENT-NUMBER: 20010008770
PGPUB-FILING-TYPE: new-utility
DOCUMENT-IDENTIFIER: US 20010008770 A1

TITLE: MICRO-ORGANISM WHICH CAN DESULPHURISE
BENZOTHIOPHENES

PUBLICATION-DATE: July 19, 2001
US-CL-CURRENT: 435/262; 435/243

APPL-NO: 09/ 230658
DATE FILED: March 24, 1999
CONTINUED PROSECUTION APPLICATION: CPA

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

APPL-NO

DOC-ID

APPL-DATE

GB

9615928.0

1996GB-9615928.0

July 30, 1996

PCT-DATA:
APPL-NO

DATE-FILED

PUB-NO

PUB-DATE

371-DATE

102(E)-DATE

PCT/GB97/02055

Jul 30, 1997

IN: OLDFIELD, CHRISTOPHER

AB: The present invention provides a micro-organism which can desulphurise benzothiophenes and a micro-organism which can desulphurise both benzothiophenes and dibenzothiophenes and a process for the production of such organisms. Such organisms and/or information derived from them can be used in devising a biocatalyst for desulphurisation of petrochemicals.

L3: Entry 1 of 28

File: PGPB

Jul 19, 2001

DOCUMENT-IDENTIFIER: US 20010008770 A1
TITLE: MICRO-ORGANISM WHICH CAN DESULPHURISE
BENZOTHIOPHENES

DETX:

[0112] Li, M. Z., Squires, C. H. and Childs, J. D. (1996) Genetic analysis of the dsz promoter and associated regulatory regions of Rhodococcus erythropolis IGTS8 J Bact 178, 6409-6418.

2. Document ID: US 6333401 B1

L3: Entry 2 of 28

File: USPT

Dec 25, 2001

US-PAT-NO: 6333401
DOCUMENT-IDENTIFIER: US 6333401 B1
TITLE: Phenol-induced proteins of Thauera aromatica
DATE-ISSUED: December 25, 2001

US-CL-CURRENT: 536/23.2; 435/252.3, 435/254.11, 435/325, 435/419

APPL-NO: 9/ 516914
DATE FILED: March 1, 2000

PARENT-CASE:

This application claims benefit of Provisional Application No. 60/122,952, filed Mar. 5, 1999.

IN: Fuchs; Georg, Breinig; Sabine

AB: This invention pertains to genes coding for phenol-induced proteins-Five phenol-induced proteins isolated from Thauera aromatica. Three dominant phenol-induced proteins called F1, F2, and F3 respectively were purified and sequenced to obtain the enzyme(s) that catalyze the .sup.14 CO.sub.2 :4-hydroxybenzoate isotope exchange reaction and the carboxylation of phenylphosphate. The N-terminal amino acid sequences of these proteins as well as the N-terminus of the phenol-induced proteins (F4 and F5) were also determined.

L3: Entry 2 of 28

File: USPT

Dec 25, 2001

DOCUMENT-IDENTIFIER: US 6333401 B1
TITLE: Phenol-induced proteins of Thauera aromatica

DEPR:

Initiation control regions or promoters, which are useful to drive expression of the instant ORF's in the desired host cell are numerous and familiar to those skilled in the art. A promoter capable of driving these genes is suitable for the present invention including but not limited to CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI (useful for expression in Saccharomyces); AOX1 (useful for expression in Pichia); and lac, trp, IP.sub.L, IP.sub.R, T7, tac, and trc (useful for expression in Escherichia coli). Useful strong promoters may also be used from Corynebacterium, Comamonas, Pseudomonas, and Rhodococcus.

3. Document ID: US 6133016 A

L3: Entry 3 of 28

File: USPT

Oct 17, 2000

US-PAT-NO: 6133016
DOCUMENT-IDENTIFIER: US 6133016 A
TITLE: Sphingomonas biodesulfurization catalyst

DATE-ISSUED: October 17, 2000

US-CL-CURRENT: 435/252.3; 435/189, 435/254.11, 435/320.1, 536/23.2

APPL-NO: 8/ 851089

DATE FILED: May 5, 1997

PARENT-CASE:

RELATED APPLICATIONS This application is a Continuation-in-part of Ser. No. 08/835,292, filed Apr.

7, 1997, now abandoned, the contents of which are incorporated herein by reference in their entirety.

IN: Darzins; Aldis, Mrachko; Gregory T.

AB: The invention relates to a novel microorganism, designated *Sphingomonas* sp. strain AD109, which is capable of selectively desulfurizing dibenzothiophene. The invention also includes isolated proteins and nucleic acid sequences obtained from this microorganism. In another embodiment, the invention provides a method of using this microorganism or enzyme preparations derived therefrom in the biocatalytic desulfurization of a fossil fuel containing organic sulfur compounds.

L3: Entry 3 of 28

File: USPT

Oct 17, 2000

DOCUMENT-IDENTIFIER: US 6133016 A

TITLE: *Sphingomonas* biodesulfurization catalyst

ORPL:

Li, M.Z. et al., "Genetic Analysis of the *dsz* Promoter and Associated Regulatory Regions of *Rhodococcus erythropolis* IGTS8," *J. Bacteriol.*, 178(22): 6409-6418 (Nov. 1996).

4. Document ID: US 6117658 A

L3: Entry 4 of 28

File: USPT

Sep 12, 2000

US-PAT-NO: 6117658

DOCUMENT-IDENTIFIER: US 6117658 A

TITLE: Methods of making polyhydroxyalkanoates comprising 4-hydroxybutyrate monomer units

DATE-ISSUED: September 12, 2000

US-CL-CURRENT: 435/135; 435/141, 435/146, 435/170

APPL-NO: 9/ 023029

DATE FILED: February 12, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATION This application claims the benefit of U.S. Provisional Application No. 60/037,758, filed Feb. 13, 1997.

IN: Dennis; Douglas E., Valentin; Henry E.

AB: The PHA biosynthetic pathway is combined with a succinic

semialdehyde metabolic

pathway that metabolizes succinic semialdehyde via a 4-hydroxybutyryl-CoA intermediate in order to produce high levels of PHA comprising 4HB monomer units. This combination is particularly useful, in part, because the PHA biosynthetic pathway is well known and has been expressed to produce P(3HB) to levels as high as 70-80% of the cell dry weight, while the succinic semialdehyde metabolic pathway is also well known, and the combination of the two pathways means that production of PHAs comprising 4HB monomer units does not depend on immediate precursors of 4-hydroxybutyrate. Rather, the production of the PHAs comprising 4HB monomer units can occur simply from the extraction of succinate or succinyl-CoA from the citric acid cycle when the host cell is grown on an inexpensive carbon source such as glucose.

L3: Entry 4 of 28

File: USPT

Sep 12, 2000

DOCUMENT-IDENTIFIER: US 6117658 A

TITLE: Methods of making polyhydroxyalkanoates comprising 4-hydroxybutyrate monomer units

BSPR:

In yet another aspect, the present invention provides a transgenic plant whose germ or somatic cells contain at least one recombinant sequence that encodes a polyhydroxyalkanoate biosynthetic pathway, and at least one sequence that encodes a succinic semialdehyde metabolic pathway that metabolizes succinic semialdehyde via a 4-hydroxybutyryl-CoA intermediate. In a preferred embodiment, the plant is *Arabidopsis thaliana*. In another preferred embodiment, the polyhydroxyalkanoate biosynthetic pathway comprises a polyhydroxyalkanoate synthase from *Nocardia corallina* pha operably linked to a promoter such that the gene is able to be expressed in the transgenic plant.

5. Document ID: US 6103508 A

L3: Entry 5 of 28

File: USPT

Aug 15, 2000

US-PAT-NO: 6103508

DOCUMENT-IDENTIFIER: US 6103508 A

TITLE: Method for removing fumarase activity, microorganisms obtainable by the method, and production of optically active aminopolycarboxylic acids using the microorganisms

DATE-ISSUED: August 15, 2000

US-CL-CURRENT: 435/184; 435/106, 435/109, 435/116, 435/128, 435/800, 435/820, 435/822, 435/830, 435/848, 435/874

APPL-NO: 9/ 179966

DATE FILED: October 28, 1998

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

APPL-NO

APPL-DATE

JP

9-311046

October 28, 1997

IN: Kato; Mami, Kaneko; Makoto, Endo; Takakazu

AB: This invention relates to a method for removing fumarase activity from a microorganism or processed product thereof having ethylenediamine-N,N'-disuccinic acid ethylenediamine lyase activity, which includes treating the microorganism or processed product thereof with an aqueous alkaline solution at a pH of 8.0 to 10.5 in the presence of at least one salt with a concentration of 5 mM to 1000 mM. The salt is preferably selected from the group consisting of sodium, potassium, ammonium and C.sub.2-6 alkanediamine salts of boric acid, phosphoric acid, hydrochloric acid, sulfuric acid, acetic acid, oxalic acid, fumaric acid, maleic acid and ethylenediamine-N,N'-disuccinic acid, and mixtures thereof. This invention also relates to a microorganism or processed product thereof having reduced fumarase activity obtainable by the above described method, and to a method for producing an optically active aminopolycarboxylic acid from fumaric acid and a compound having amino group in the presence of the microorganism or processed product thereof having reduced fumarase activity as a catalyst.

L3: Entry 5 of 28

File: USPT

Aug 15, 2000

DOCUMENT-IDENTIFIER: US 6103508 A

TITLE: Method for removing fumarase activity, microorganisms obtainable by the method, and production of optically active aminopolycarboxylic acids using the microorganisms

DEPR:

To 2 .mu.l of plasmid pEDS020 obtained in Step (10), 2 .mu.l of 10.times. restriction enzyme reaction buffer, 15 .mu.l of sterilized water, 1 .mu.l of restriction enzyme XhoI are added, and the mixture is reacted at 37.degree. C. for 2 hours. The plasmid is recovered by ethanol precipitation and dried, to which 15 .mu.l of sterilized water, 2 .mu.l of a 10.times. klenow fragment buffer, 2 .mu.l of a 10 mM dNTP's solution and 1 .mu.l of a klenow fragment are added, followed by 2-hour reaction at 37.degree. C. The DNA fragment is recovered by ethanol precipitation and dried, to which are added 8 .mu.l of sterilized water, 1 .mu.l of XbaI linker solution, and 16 .mu.l of Solution A and 4 .mu.l of Solution B both from Ligation kit (Takara Shuzo Co., Ltd.). The mixture is reacted at 16.degree. C. for 4 hours. After transformation into JM109, a plasmid is obtained from the transformant, the plasmid having a change of the XhoI site of pEDS020 to XbaI site. To 2 .mu.l of the obtained plasmid are added 2 .mu.l of 10.times. restriction enzyme reaction buffer, 15 .mu.l of sterilized water and 1 .mu.l of restriction enzyme EcoRV, and the mixture is reacted at 37.degree. C. for 2 hours. The DNA fragment is recovered by ethanol precipitation and dried. Then, 8 .mu.l of sterilized water, 1 .mu.l of Sse8387I linker solution, and 16 .mu.l of Solution A and 4 .mu.l of Solution B both from Ligation kit (Takara Shuzo Co., Ltd.) are added to the dried DNA, and the

mixture is reacted at 16.degree. C. for 4 hours and transformed into JM109. From the transformant, the plasmid pEDS027 is obtained which has a change of generally EcoRV site to Sse8387I site. To 2 .mu.l of the thus-obtained plasmid are added 2 .mu.l of 10.times. restriction enzyme reaction buffer, 14 .mu.l of sterilized water, 1 .mu.l of restriction enzyme XbaI and 1 .mu.l of Sse8387I, and the mixture is reacted at 37.degree. C. for 2 hours. A 1.7 Kb band is separated from the reaction mixture by agarose gel electrophoresis, which is then inserted into the XbaI-Sse8387I site of plasmid pSJ034 having a strong Rhodococcus promoter activity, thereby producing the transformant plasmid pSE001 (FIG. 5). The plasmid pSJ034 is prepared from the plasmid pSJ023 (Japanese Patent Application No. 9-65618) by the procedure shown in FIG. 6. The plasmid pSJ023 was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, MITI (Japan) as transformant Rhodococcus rhodochrous ATCC12674/pSJ023 (FERM P-16108) on Mar. 4, 1997 which was subsequently transferred to the international deposition as FERM BP-6232 on Jan. 21, 1998.

6. Document ID: US 6071738 A

L3: Entry 6 of 28

File: USPT

Jun 6, 2000

US-PAT-NO: 6071738

DOCUMENT-IDENTIFIER: US 6071738 A

TITLE: Conversion of organosulfur compounds to oxyorganosulfur compounds for desulfurization of fossil fuels
DATE-ISSUED: June 6, 2000

US-CL-CURRENT: 435/282; 435/130, 435/281

APPL-NO: 8/ 933885

DATE FILED: September 19, 1997

IN: Johnson; Steven W., Monticello; Daniel J., Hazan; Charles, Colin; Jean-Michel

AB: The present invention relates to a method for the desulfurization of a fossil fuel containing one or more organosulfur compounds. In one embodiment, the method comprises the steps of (1) contacting the fossil, fuel with a biocatalyst capable of converting the organosulfur compound to an oxyorganosulfur compound which is separable from the fossil fuel; and (2) separating the oxyorganosulfur compound from the fossil fuel. The oxyorganosulfur compound can then be isolated, discarded or further processed, for example, via desulfurization by a biocatalyzed process or an abiotic process, such as hydrodesulfurization.

L3: Entry 6 of 28

File: USPT

Jun 6, 2000

DOCUMENT-IDENTIFIER: US 6071738 A

TITLE: Conversion of organosulfur compounds to oxyorganosulfur

compounds for desulfurization of
fossil fuels

File: USPT

Mar 9, 1999

ORPL:

Li, M.Z. et al., "Genetic Analysis of the dsz Promoter and Associated
Regulatory Regions of
Rhodococcus erythropolis IGTS8," J. Bacteriol., 178(22): 6409-6418
(Nov. 1996).

US-PAT-NO: 5879914

DOCUMENT-IDENTIFIER: US 5879914 A

TITLE: Recombinant DNA encoding a desulfurization biocatalyst
DATE-ISSUED: March 9, 1999

US-CL-CURRENT: 435/130; 435/189, 435/232, 435/252.3, 435/320.1,
536/23.2

APPL-NO: 8/ 421791

DATE FILED: April 14, 1995

PARENT-CASE:

RELATED APPLICATION This application is a division of application
Ser. No. 08/304,081 filed Sep. 1,
1994, which is a Divisional of Ser. No. 08/089,755 filed Jul. 9, 1993 now
U.S. Pat. No. 5,356,801,
which is a Continuation-In-Part of Ser. No. 07/911,845 filed Jul. 10, 1992
now abandoned.

IN: Rambosek; John, Piddington; Chris S., Kovacevich; Brian R.,
Young; Kevin D., Denome;
Sylvia A.

AB: This invention relates to recombinant DNA molecule containing
a gene or genes which
encode a biocatalyst capable of desulfurizing a fossil fuel which contains
organic sulfur
molecules. For example, the present invention encompasses a
recombinant DNA molecule containing
a gene or genes of a strain of Rhodococcus rhodochrous.

7. Document ID: US 5985560 A

L3: Entry 7 of 28

File: USPT

Nov 16, 1999

US-PAT-NO: 5985560

DOCUMENT-IDENTIFIER: US 5985560 A

TITLE: Cloning vector and a process for the preparation thereof
DATE-ISSUED: November 16, 1999

US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 435/440, 435/69.1,
435/91.4, 435/91.41, 435/91.42

APPL-NO: 8/ 853097

DATE FILED: May 8, 1997

IN: Lal; Rup

AB: This invention relates to a method for the preparation of a series
of cloning
vectors and such cloning vectors prepared therefrom. The method
consists in the step of
digestion of pRL1 derivative with a restriction enzyme BamHI and
digesting the pJ4026 by BglII
and electroeluting the ermE gene. The linear DNA having pRL1
derivative is ligated with ermE
gene and then transformed into E. coli GM2163. The transformants are
screened for the presence
of concatamers and that concatamers of pRL50 and pRL80 DNAs being
isolated from E. coli. The
transformants are selected under appropriate antibiotic selection pressure.

L3: Entry 7 of 28

File: USPT

Nov 16, 1999

DOCUMENT-IDENTIFIER: US 5985560 A

TITLE: Cloning vector and a process for the preparation thereof

ORPL:

Kumar et al. Efficient transformation of the cephamycin C producer
Nocardia lactamdurans and
development of shuttle and promoter-probe cloning vectors. Applied and
Environmental Microbiology
vol. 60 pp. 4086-4093, 1994.

8. Document ID: US 5879914 A

L3: Entry 8 of 28

L3: Entry 8 of 28

File: USPT

Mar 9, 1999

DOCUMENT-IDENTIFIER: US 5879914 A

TITLE: Recombinant DNA encoding a desulfurization biocatalyst

DEPR:

Amplification yielded the predicted 2846 bp fragment. In order to express
the amplified fragment
harboring ORFs 2 and 3, it was ligated to the XbaI/EcoRI fragment of the
chloramphenicol resistance
gene promoter from Rhodococcus fascians (Desomer et al.: Molecular
Microbiology (1992) 6 (16),
2377-2385) to give plasmid pOTTO-1. Ultimately, a blunt end ligation was
used for the subcloning of
the amplified product due to the fact that ligation using the engineered
restriction sites was
unsuccessful. This fusion was ligated to shuttle-vector pRR-6 to produce
plasmid pENOK-20. CPE648
transformants of pENOK-20 were grown in the presence of DBT and 25
Mg/ml chloramphenicol for
promoter induction. All transformants converted DBT to DBT-sulfone
presumably through the activity
of the ORF 3 as demonstrated in subclone pENOK-3. The inability to
further process DBT-sulfone with
the presence of ORF 2 suggests that the product of ORF 2 alone is
incapable of using DBT-sulfone as
a substrate. This is consistent with results obtained from pENOK-Nsi, and
suggests that ORF-2 alone
is incapable of using DBT-sulfone as a substrate.

DEPR:

Increasing the specific activity of desulfurization is a significant objective
of the studies
described herein. One approach to accomplishing this goal is to replace the
original promoter with
one that can produce both higher and constitutive expression of the
desulfurization gene cluster.
Because there are so few reported and characterized Rhodococcus

promoters, random genomic libraries have been prepared and screened for promoter activity in two systems. In one, the reporter is the chloramphenicol resistance gene used in the above-discussed plasmid constructions. In the other, the desulfurization cluster itself is used as a reporter.

DEPR:

As also described below, partially digested *Rhodococcus* genomic DNA has been cloned upstream of a promoterless chloramphenicol resistance gene. The resulting libraries were then transformed into *Rhodococcus* which are subjected to chloramphenicol selection. Four apparent promoter elements were rescued by pRHODOPRO-2, although plasmid could be isolated from only one of these, possibly due to vector instability. The stable plasmid RP2-2A has been subjected to sequence analysis. Technical problems have been observed with restriction enzyme treatment of the NarI cloning site used in these vectors. Unfortunately, the NarI enzyme demonstrates severe site-selectivity and does not appear to digest the vector well. New vectors have been constructed in order to alleviate this problem, although a lack of convenient and unique restriction sites slowed the progress of these studies. A recent observation on the *Rhodococcus* replication origin will aid in constructing a more effective promoter probe, as discussed below.

DEPR:

Examples of potential alternative promoters include other known and described promoters such as the chloramphenicol resistance gene promoter from *Rhodococcus fascians* (Desomer et al.: *Molecular Microbiology* (1992) 6 (16), 2377-2385), the nitrile hydratase gene promoter from *Rhodococcus rhodochrous* (Kobayashi, et al.: *Biochimica et Biophysica Acta*, 1129 (1991) 23-33), or other strong promoters isolated from *Rhodococcus* sp. by "shot-gun" promoter probing. Other potential alternative promoters include those from other Gram positive organisms such as *Corynebacterium*, *Bacillus*, *Streptomyces*, and the like.

DEPR:

Each ORF has been successfully amplified and subcloned into pUC-19 NdeI as EcoRI fragments. Alternative promoters will be ligated into the unique NdeI sites, and the fusions will be moved to *Rhodococcus-E. coli* shuttle vector pRR-6 for expression in *Rhodococcus*.

DEPL:

Expression from the chloramphenicol resistance gene promoter from *Rhodococcus fascians*.

9. Document ID: US 5830693 A

L3: Entry 9 of 28

File: USPT

Nov 3, 1998

US-PAT-NO: 5830693

DOCUMENT-IDENTIFIER: US 5830693 A

TITLE: Gene encoding a regulatory factor involved in activating expression of the nitrilase gene promoter

DATE-ISSUED: November 3, 1998

US-CL-CURRENT: 435/69.1; 435/136, 435/169, 435/227, 435/252.3, 435/320.1, 435/71.2, 530/350,

536/23.1, 536/23.74

APPL-NO: 8/ 683908

DATE FILED: July 19, 1996

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

APPL-NO

APPL-DATE

JP

7-185626

July 21, 1995

IN: Shimizu; Sakayu, Kobayashi; Michihiko

AB: The invention relates to a regulatory factor substantially containing an amino acid sequence represented by SEQ ID NO:1 and having the action of activating a nitrilase gene promoter, a regulatory factor gene containing DNA coding substantially for said regulatory factor, a recombinant plasmid containing said regulatory factor gene, a nitrilase gene containing a promoter region and a DNA region capable of replicating in a microorganism belonging to the genus *Rhodococcus*, and a transformant transformed with said recombinant plasmid.

L3: Entry 9 of 28

File: USPT

Nov 3, 1998

DOCUMENT-IDENTIFIER: US 5830693 A

TITLE: Gene encoding a regulatory factor involved in activating expression of the nitrilase gene promoter

ABPL:

The invention relates to a regulatory factor substantially containing an amino acid sequence represented by SEQ ID NO:1 and having the action of activating a nitrilase gene promoter, a regulatory factor gene containing DNA coding substantially for said regulatory factor, a recombinant plasmid containing said regulatory factor gene, a nitrilase gene containing a promoter region and a DNA region capable of replicating in a microorganism belonging to the genus *Rhodococcus*, and a transformant transformed with said recombinant plasmid.

BSPR:

The present invention relates to a regulatory factor involved in expression of a nitrilase gene as well as to DNA coding for said regulatory factor. In particular, the present invention relates to a regulatory factor derived from *Rhodococcus rhodochrous* J1 and having the action of activating a nitrilase gene promoter, a recombinant plasmid containing DNA coding for said regulatory factor, a nitrilase gene promoter and a nitrilase gene, and a transformant transformed with said recombinant plasmid.

BSPR:

In order to prepare a microorganism as a catalyst having higher catalytic activity, the present inventors cloned a nitrilase gene from *Rhodococcus rhodochrous* J1 and constructed a plasmid by inserting the gene to a region downstream of *E. coli* lactose promoter (*J. Biol. Chem.* 267, 20746-20751 (1992)). *E. coli* into which said plasmid had been introduced exhibited higher nitrilase

activity when cultured in the presence of IPTG
(isopropyl-.beta.-D-thiogalactoside).

BSPR:

The object of the present invention is to provide a regulatory factor derived from *Rhodococcus* rhodochrous J1 and having the action of activating a nitrilase gene promoter, a recombinant plasmid containing DNA coding for said regulatory factor, a nitrilase gene promoter and a nitrilase gene, and a transformant transformed with said recombinant plasmid.

BSPR:

The present inventors speculated that the reason the gene is not expressed by the transformant derived from the genus *Rhodococcus* is that the promoter for the nitrilase gene fails to function because the transformant does not carry a gene coding for a regulatory factor necessary for functioning of the promoter. Hence, they thought that a gene coding for such regulatory factor is present somewhere in chromosomal DNA from the J1 strain and found the gene located downstream of the nitrilase structural gene. As a result of preparation of a transformant belonging to the genus *Rhodococcus* carrying this gene, the transformant could successfully express nitrilase with high activity.

BSPR:

Further, the present invention is a recombinant plasmid containing said regulatory factor gene, a nitrilase gene containing a promoter region, and a DNA region capable of replicating in a microorganism belonging to the genus *Rhodococcus*. As the DNA region capable of replicating in a microorganism belonging to the genus *Rhodococcus*, mention may be made of a member selected from a group consisting of plasmids pRC001, pRC002, pRC003 and pRC004.

CLPR:

11. A method of activating a nitrilase gene promoter for expression of nitrilase enzyme in a *Rhodococcus* host cell the method comprising transforming said host cell with the plasmid of any one of claims 6, 7 or 8 culturing the transformed cells in the presence of isovaleronitrile such that the regulatory factor is expressed and activates the nitrilase gene promoter resulting in expression of the nitrilase enzyme in the host cell.

10. Document ID: US 5683913 A

L3: Entry 10 of 28

File: USPT

Nov 4, 1997

US-PAT-NO: 5683913

DOCUMENT-IDENTIFIER: US 5683913 A

TITLE: Regulatory gene for the expression of nitrile hydratase gene

DATE-ISSUED: November 4, 1997

US-CL-CURRENT: 435/252.3; 435/254.11, 435/320.1, 435/325, 536/23.7

APPL-NO: 8/ 537434

DATE FILED: October 2, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY

APPL-NO

APPL-DATE

JP

6-239263

October 3, 1994

IN: Shimizu; Sakayu, Kobayashi; Michihiko

AB: The invention relates to a regulatory gene coding for a polypeptide having the ability to activate a promoter for a nitrile hydratase gene, a recombinant DNA containing said regulatory gene, and a transformant transformed with said recombinant DNA. The introduction of the regulatory gene of the invention along with a nitrile hydratase gene and its promoter region permits bacteria of the genus *Rhodococcus* to produce a higher level of nitrile hydratase. Other extraneous gene can also be introduced into a region downstream of the promoter to produce other proteins in high yield.

L3: Entry 10 of 28

File: USPT

Nov 4, 1997

DOCUMENT-IDENTIFIER: US 5683913 A

TITLE: Regulatory gene for the expression of nitrile hydratase gene

ABPL:

The introduction of the regulatory gene of the invention along with a nitrile hydratase gene and its promoter region permits bacteria of the genus *Rhodococcus* to produce a higher level of nitrile hydratase. Other extraneous gene can also be introduced into a region downstream of the promoter to produce other proteins in high yield.

BSPR:

The present invention relates to a regulatory gene derived from bacteria of the genus *Rhodococcus* and coding for a polypeptide capable of activating a promoter for a nitrile hydratase gene, a recombinant DNA containing said DNA, and a transformant transformed with said recombinant DNA.

BSPR:

That is, the present invention relates to a regulatory gene derived from bacteria of the genus *Rhodococcus* and coding for a polypeptide capable of activating a promoter for the nitrile hydratase gene, a recombinant DNA containing said DNA, and a transformant transformed with said recombinant DNA.

DEPR:

The introduction of the regulatory gene of the invention along with the nitrile hydratase gene and its promoter region permits bacteria of the genus *Rhodococcus* to produce a higher level of nitrile hydratase. Other extraneous gene can also be introduced into a region downstream of the promoter to produce other proteins in high yield.

11. Document ID: US 5602014 A

L3: Entry 11 of 28

File: USPT

Feb 11, 1997

US-PAT-NO: 5602014
DOCUMENT-IDENTIFIER: US 5602014 A
TITLE: Regulatory system for expression of nitrilase gene
DATE-ISSUED: February 11, 1997

US-CL-CURRENT: 435/129; 435/252.3, 435/320.1, 435/69.1, 536/23.7

APPL-NO: 8/ 577184
DATE FILED: December 22, 1995

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY

APPL-NO	APPL-DATE
JP	
6-337652	December 28, 1994

IN: Mizumura; Yurie, Yu; Fujio

AB: The invention relates to a regulatory factor or system composed of 2 components of a polypeptide having the amino acid sequence of SEQ ID No:1 and a polypeptide having the amino acid sequence of SEQ ID No:2 to activate a nitrilase gene promoter, as well as to DNA coding therefor. Nitrilase can be produced by introducing the gene coding for the present regulatory factor together with a nitrilase gene containing a promoter region into a microorganism of the genus *Rhodococcus*.

L3: Entry 11 of 28

File: USPT

Feb 11, 1997

DOCUMENT-IDENTIFIER: US 5602014 A
TITLE: Regulatory system for expression of nitrilase gene

ABPL:

The invention relates to a regulatory factor or system composed of 2 components of a polypeptide having the amino acid sequence of SEQ ID No:1 and a polypeptide having the amino acid sequence of SEQ ID No:2 to activate a nitrilase gene promoter, as well as to DNA coding therefor. Nitrilase can be produced by introducing the gene coding for the present regulatory factor together with a nitrilase gene containing a promoter region into a microorganism of the genus *Rhodococcus*.

BSPR:

The present invention relates to a regulatory factor involved in expression of a nitrilase gene and a DNA coding for the same and particularly to a regulatory factor derived from the strain *Rhodococcus erythropolis* SK92 and activating a nitrilase gene promoter, as well as to DNAs coding for the same, a recombinant plasmid containing the DNAs and a transformant transformed with said recombinant plasmid.

BSPR:

As compared with the above-mentioned conventional processes, the use of a nitrilase gene cloned for hydrolysis of nitriles by genetic recombination is expected to drastically improve the catalytic ability of the microorganism to hydrate nitriles because the microorganism can be engineered to contain multiple copies of the same gene. To obtain such a catalyst organism with higher catalytic

activity, the present inventors successfully cloned a nitrilase gene from the strain *Rhodococcus erythropolis* SK92 and constructed a plasmid by inserting said gene into a region downstream of an E.

coli lactose promoter. By introducing this plasmid into *E. coli*, the organism came to exhibit higher nitrilase activity during incubation in the presence of IPTG (isopropyl-beta.-D-thiogalactoside).

The present inventors further attempted to obtain a transformant of the genus *Rhodococcus* to attain higher performance as a catalyst organism. In this attempt, the nitrilase gene was inserted into a

Rhodococcus-E. coli hybrid plasmid vector (see Japanese Laid-Open Patent Publication Nos. 4,589/1993 and 68,566/1993), and the vector thus constructed was introduced into a microorganism of the genus

Rhodococcus. However, no nitrilase activity was expressed, and there is demand for a method of

permitting the expression of nitrilase activity in a transformant of the genus *Rhodococcus*.

BSPR:

The present inventors speculated that the gene derived from the genus *Rhodococcus* is not expressed because the promoter for the nitrilase gene fails to function, and that a gene coding for a

regulatory factor that allows the promoter to function might be present somewhere on the chromosomal

DNA derived from SK92. Through screening, the present inventors found it in a region upstream of the

nitrilase structural gene and succeeded thereby in expression of nitrilase activity in a transformant of the genus *Rhodococcus*.

BSPR:

Introduction of the gene or genes coding for the regulatory factor of the invention along with the nitrilase gene containing its promoter permits microorganism of the genus *Rhodococcus* to produce nitrilase.

12. Document ID: US 5578478 A

L3: Entry 12 of 28

File: USPT

Nov 26, 1996

US-PAT-NO: 5578478
DOCUMENT-IDENTIFIER: US 5578478 A
TITLE: Recombinant DNA encoding a desulfurization biocatalyst
DATE-ISSUED: November 26, 1996

US-CL-CURRENT: 435/195; 435/252.3, 435/69.1, 536/22.1, 536/23.1, 536/23.2, 536/23.7

APPL-NO: 8/ 421754
DATE FILED: April 14, 1995

PARENT-CASE:

This application is a division of application Ser. No. 08/304,081 filed Sep. 1, 1994 which is a divisional of 08/089,755 filed Jul. 9, 1993 now U.S. Pat. No. 5,356,801 which is a continuation-in-part of 07/911,845, now abandoned filed Jul. 10, 1992.

IN: Rambosek; John, Piddington; Chris S., Kovacevich; Brian R., Young; Kevin D., Denome; Sylvia A.

AB: This invention relates to a recombinant DNA molecule

containing a gene or genes
which encode a biocatalyst capable of desulfurizing a fossil fuel which
contains organic sulfur
molecules. For example, the present invention encompasses a
recombinant DNA molecule containing
a gene or genes of a strain of *Rhodococcus rhodochrous*.

L3: Entry 12 of 28

File: USPT

Nov 26, 1996

DOCUMENT-IDENTIFIER: US 5578478 A

TITLE: Recombinant DNA encoding a desulfurization biocatalyst

DEPR:

Amplification yielded the predicted 2846 bp fragment. In order to express
the amplified fragment
harboring ORFs 2 and 3, it was ligated to the XbaI/EcoRI fragment of the
chloramphenicol resistance
gene promoter from *Rhodococcus fascians* (Desomer et al.: Molecular
Microbiology (1992) 6 (16),
2377-2385) to give plasmid pOTTO-1. Ultimately, a blunt end ligation was
used for the subcloning of
the amplified product due to the fact that ligation using the engineered
restriction sites was
unsuccessful. This fusion was ligated to shuttle-vector pRR-6 to produce
plasmid pENOK-20. CPE648
transformants of pENOK-20 were grown in the presence of DBT and 25
.mu.g/ml chloramphenicol for
promoter induction. All transformants converted DBT to DBT-sulfone
presumably through the activity
of the ORF 3 as demonstrated in subclone pENOK-3. The inability to
further process DBT-sulfone with-
the presence of ORF 2 suggests that the product of ORF 2 alone is
incapable of using DBT-sulfone as
a substrate. This is consistent with results obtained from pENOK-Nsi, and
suggests that ORF-2 alone
is incapable of using DBT-sulfone as a substrate.

DEPR:

Increasing the specific activity of desulfurization is a significant objective
of the studies
described herein. One approach to accomplishing this goal is to replace the
original promoter with
one that can produce both higher and constitutive expression of the
desulfurization gene cluster.

Because there are so few reported and characterized *Rhodococcus*
promoters, random genomic libraries
have been prepared and screened for promoter activity in two systems. In
one, the reporter is the
chloramphenicol resistance gene used in the above-discussed plasmid
constructions. In the other, the
desulfurization cluster itself is used as a reporter.

DEPR:

As also described below, partially digested *Rhodococcus* genomic DNA
has been cloned upstream of a
promoterless chloramphenicol resistance gene. The resulting libraries were
then transformed into
Rhodococcus which are subjected to chloramphenicol selection. Four
apparent promoter elements were
rescued by pRHODOPRO-2, although plasmid could be isolated from only
one of these, possibly due to
vector instability. The stable plasmid RP2-2A has been subjected to
sequence analysis. Technical
problems have been observed with restriction enzyme treatment of the NarI
cloning site used in these
vectors. Unfortunately, the NarI enzyme demonstrates severe
site-selectivity and does not appear to
digest the vector well. New vectors have been constructed in order to
alleviate this problem,
although a lack of convenient and unique restriction sites slowed the
progress of these studies. A
recent observation on the *Rhodococcus* replication origin will aid in
constructing a more effective

promoter probe, as discussed below.

DEPR:

Examples of potential alternative promoters include other known and
described promoters such as the
chloramphenicol resistance gene promoter from *Rhodococcus fascians*
(Desomer et al.: Molecular
Microbiology (1992) 6 (16), 2377-2385), the nitrile hydratase gene
promoter from *Rhodococcus*
rhodochrous (Kobayashi, et al.: Biochimica et Biophysica Acta, 1129
(1991) 23-33), or other strong
promoters isolated from *Rhodococcus* sp. by "shot-gun" promoter probing.
Other potential alternative
promoters include those from other Gram positive organisms such as
Corynebacterium, *Bacillus*,
Streptomyces, and the like.

DEPR:

Promoter Engineering Example A: Expression from the chloramphenicol
resistance gene promoter from
Rhodococcus fascians.

DEPR:

Each ORF has been successfully amplified and subcloned into pUC-19
NdeI as EcoRI fragments.
Alternative promoters will be ligated into the unique NdeI sites, and the
fusions will be moved to
Rhodococcus-E. coli shuttle vector pRR-6 for expression in *Rhodococcus*.

13. Document ID: US 5371006 A

L3: Entry 13 of 28

File: USPT

Dec 6, 1994

US-PAT-NO: 5371006

DOCUMENT-IDENTIFIER: US 5371006 A

TITLE: Isolated DNA encoding the NotI restriction endonuclease and
related methods for producing the
same
DATE-ISSUED: December 6, 1994

US-CL-CURRENT: 435/199; 435/193, 435/196, 435/252.33, 435/320.1,
536/23.2

APPL-NO: 8/ 003254

DATE FILED: January 11, 1993

IN: Morgan; Richard D., Benner; Jack S., Claus; Toby E.

AB: The present invention is directed to a method for cloning and
producing the NotI
restriction endonuclease by 1) introducing the restriction endonuclease
gene from *Nocardia*
otitidis-caviae into a host whereby the restriction gene is expressed; 2)
fermenting the host
which contains the plasmid encoding and expressing the NotI restriction
endonuclease activity,
and 3) purifying the NotI restriction endonuclease from the fermented
host which contains the
plasmid encoding and expressing the NotI restriction endonuclease
activity.

L3: Entry 13 of 28

File: USPT

Dec 6, 1994

DOCUMENT-IDENTIFIER: US 5371006 A

TITLE: Isolated DNA encoding the NotI restriction endonuclease and related methods for producing the same

DEPV:

10. Overexpressing the NotI endonuclease gene: There are a number of ways in which the clone containing the restriction gene can be overexpressed. The DNA sequence and detailed mapping help determine the best approach for overexpression of the restriction endonuclease gene. One approach for overexpression comprises designing primers that hybridize directly at the N-terminus of the restriction endonuclease gene and somewhere downstream of the restriction endonuclease gene in order to use the polymerase-chain reaction to amplify the entire restriction endonuclease gene. The resulting DNA fragment can be cleaved to remove all Nocardia DNA preceding the endonuclease gene and can be inserted into an expression vector such as pSYX22 directly downstream of an inducible promoter such as T7. Alternatively, overexpression can be accomplished by inserting a promoter recognized strongly by E. coli, such as P.sub.tac on pAGR3 (available from New England Biolabs) directly in front of the beginning of the restriction endonuclease gene. This may be accomplished by finding convenient restriction sites near the beginning and end of the restriction endonuclease gene and compatible restriction sites near the promoter of pAGR3, and transferring the restriction gene into pAGR3 in line with the P.sub.tac promoter. Other regulated promoters which can be used are PlacUV5 (Fuller, Gene 19:43-54, (1982)), and .lambda.PL (Shimatake and Rosenberg, Nature 254:128, (1981)) on pUC19 and pBR322 derivatives. In addition, a strong ribosome binding site (Shine & Dalgarno, Proc. Natl. Acad. Sci. USA 71, 1342-1346, (1974)) can be placed in front of the gene to increase expression.

14. Document ID: US 5356801 A

L3: Entry 14 of 28

File: USPT

Oct 18, 1994

US-PAT-NO: 5356801

DOCUMENT-IDENTIFIER: US 5356801 A

TITLE: Recombinant DNA encoding a desulfurization biocatalyst

DATE-ISSUED: October 18, 1994

US-CL-CURRENT: 435/195; 435/252.3, 435/320.1, 435/69.1, 536/22.1, 536/23.1, 536/23.2, 536/23.7

APPL-NO: 8/089755

DATE FILED: July 9, 1993

PARENT-CASE:

RELATED APPLICATION This application is a continuation-in-part application of U.S. Ser. No.

07/911,845, now abandoned, filed with the United States Patent and Trademark Office on Jul. 10, 1992.

IN: Rambosek; John, Piddington; Chris S., Kovacevich; Brian R., Young; Kevin D., Denome; Sylvia A.

AB: This invention relates to a recombinant DNA molecule containing a gene or genes which encode a biocatalyst capable of desulfurizing a fossil fuel which contains organic sulfur molecules. For example, the present invention encompasses a recombinant DNA molecule containing a gene or genes of a strain of Rhodococcus rhodochrous.

L3: Entry 14 of 28

File: USPT

Oct 18, 1994

DOCUMENT-IDENTIFIER: US 5356801 A

TITLE: Recombinant DNA encoding a desulfurization biocatalyst

DEPR:

Amplification yielded the predicted 2846 bp fragment. In order to express the amplified fragment harboring ORFs 2 and 3, it was ligated to the XbaI/EcoRI fragment of the chloramphenicol resistance gene promoter from Rhodococcus fascians (Desomer et al.: Molecular Microbiology (1992) 6 (16), 2377-2385) to give plasmid pOTTO-1. Ultimately, a blunt end ligation was used for the subcloning of the amplified product due to the fact that ligation using the engineered restriction sites was unsuccessful. This fusion was ligated to shuttle-vector pRR-6 to produce plasmid pENOK-20. CPE648 transformants of pENOK-20 were grown in the presence of DBT and 25 .mu.g/ml chloramphenicol for promoter induction. All transformants converted DBT to DBT-sulfone presumably through the activity of the ORF 3 as demonstrated in subclone pENOK-3. The inability to further process DBT-sulfone with the presence of ORF 2 suggests that the product of ORF 2 alone is incapable of using DBT-sulfone as a substrate. This is consistent with results obtained from pENOK-Nsi, and suggests that ORF-2 alone is incapable of using DBT-sulfone as a substrate.

DEPR:

Increasing the specific activity of desulfurization is a significant objective of the studies described herein. One approach to accomplishing this goal is to replace the original promoter with one that can produce both higher and constitutive expression of the desulfurization gene cluster.

Because there are so few reported and characterized Rhodococcus promoters, random genomic libraries have been prepared and screened for promoter activity in two systems. In one, the reporter is the chloramphenicol resistance gene used in the above-discussed plasmid constructions. In the other, the desulfurization cluster itself is used as a reporter.

DEPR:

As also described below, partially digested Rhodococcus genomic DNA has been cloned upstream of a promoterless chloramphenicol resistance gene. The resulting libraries were then transformed into Rhodococcus which are subjected to chloramphenicol selection. Four apparent promoter elements were rescued by pRHODOPRO-2, although plasmid could be isolated from only one of these, possibly due to vector instability. The stable plasmid RP2-2A has been subjected to sequence analysis. Technical problems have been observed with restriction enzyme treatment of the NarI cloning site used in these vectors. Unfortunately, the NarI enzyme demonstrates severe site-selectivity and does not appear to digest the vector well. New vectors have been constructed in order to alleviate this problem, although a lack of convenient and unique restriction sites slowed the progress of these studies. A recent observation on the Rhodococcus replication origin will aid in

constructing a more effective promoter probe, as discussed below.

DEPR:

Examples of potential alternative promoters include other known and described promoters such as the chloramphenicol resistance gene promoter from *Rhodococcus fascians* (Desomer et al.: *Molecular Microbiology* (1992) 6 (16), 2377-2385), the nitrile hydratase gene promoter from *Rhodococcus rhodochrous* (Kobayashi, et al.: *Biochimica et Biophysica Acta*, 1129 (1991) 23-33), or other strong promoters isolated from *Rhodococcus* sp. by "shot-gun" promoter probing. Other potential alternative promoters include those from other Gram positive organisms such as *Corynebacterium*, *Bacillus*, *Streptomyces*, and the like.

DEPR:

Each ORF has been successfully amplified and subcloned into pUC-19 NdeI as EcoRI fragments. Alternative promoters will be ligated into the unique NdeI sites, and the fusions will be moved to *Rhodococcus*-E. coli shuttle vector pRR-6 for expression in *Rhodococcus*.

DEPC:

Promoter Engineering Example A: Expression from the Chloramphenicol Resistance Gene Promoter from *Rhodococcus fascians*

15. Document ID: US 4952500 A

L3: Entry 15 of 28

File: USPT

Aug 28, 1990

US-PAT-NO: 4952500

DOCUMENT-IDENTIFIER: US 4952500 A

TITLE: Cloning systems for *Rhodococcus* and related bacteria

DATE-ISSUED: August 28, 1990

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/486, 435/826, 435/863, 435/872, 435/886, 435/91.41

APPL-NO: 7/ 151319

DATE FILED: February 1, 1988

IN: Finnerty; William R., Singer; Mary E.

AB: A plasmid transformation system for *Rhodococcus* was developed using an *Escherichia coli*-*Rhodococcus* shuttle plasmid. *Rhodococcus* sp. H13-A contains three cryptic indigenous plasmids, designated pMVS100, pMVS200 and pMVS300, of 75, 19.5 and 13.4 kilobases (Kb), respectively. A 3.8 Kb restriction fragment of pMVS300 was cloned into pIJ30, a 6.3 Kb pBR322 derivative, containing the E. coli origin of replication (ori) and ampicillin resistance determinant (bla) as well as a *Streptomyces* gene for thiostrepton resistance, *tsr*. The resulting 10.1 Kb recombinant plasmid, designated pMVS301, was isolated from E. coli DH1 (pMVS301) and transformed into *Rhodococcus* sp. AS-50, a derivative of strain H13-A, by polyethylene glycol-assisted transformation of *Rhodococcus* protoplasts and selection for thiostrepton-resistant transformants. This strain was deposited with the ATCC on Feb. 1, 1988

and assigned ATCC 53719. The plasmid contains the *Rhodococcus* origin of replication. The plasmid and derivatives thereof can therefore be used to introduce nucleic acid sequences to and from *Rhodococcus* for subsequent expression and translation into protein. The isolated origin of replication can also be used in the construction of new vectors.

L3: Entry 15 of 28

File: USPT

Aug 28, 1990

DOCUMENT-IDENTIFIER: US 4952500 A

TITLE: Cloning systems for *Rhodococcus* and related bacteria

DEPR:

Rhodococcus transformants containing pMVS302 exhibit higher levels of ampicillin resistance and .beta.-lactamase activity than those containing pMVS301. The two plasmids differ only in the orientation of the cloned fragment of *Rhodococcus* DNA located upstream of the bla gene. Expression of the bla gene in E. coli transformants is independent of the orientation of the cloned *Rhodococcus* DNA, with similar levels of ampicillin resistance and .beta.-lactamase activity in transformants containing either plasmid. The orientation-dependent expression of the E. coli bla gene in *Rhodococcus* indicates that bla gene transcription occurs from a *Rhodococcus* promoter in the cloned fragment rather than from its own promoter. Alternatively, pMVS302 may have a higher copy number than pMVS301 in *Rhodococcus*. This is unlikely since both plasmids contain the same origin of replication. In addition, estimates of plasmid copy number gels of total DNA preparations indicate that pMVS301 and pMVS302 have similar copy numbers in *Rhodococcus*.

DEPR:

.beta.-lactamase activity was measured in the cells and extracellular growth medium of E. coli and *Rhodococcus* transformants to correlate levels of ampicillin resistance with .beta.-lactamase activity. .beta.-lactamase activity was exclusively extracellular in *Rhodococcus* transformants, with no detectable cell-associated activity, demonstrated in Table 5. Cell-free extracts prepared by sonication of *Rhodococcus* cells showed no detectable .beta.-lactamase activity. In E. coli transformants, 70% of the .beta.-lactamase activity was cell-associated and 30% was present in the growth medium. The specific activity of .beta.-lactamase in pMVS301-and pMVS302-containing transformants was 3-fold higher than that of the host strain, E. coli DH1. Total .beta.-lactamase activity was 7- and 47-fold higher in E. coli transformants containing pMVS301 and pMVS302, respectively, than in the corresponding *Rhodococcus* transformants, indicating lower levels of bla gene expression in *Rhodococcus* than in E. coli. The relative .beta.-lactamase activity in *Rhodococcus* strains correlated directly with the level of ampicillin resistance in the respective transformants. The expression of the E. coli bla gene in *Rhodococcus* appears dependent on the orientation of the 3.8 Kb HindIII *Rhodococcus* DNA fragment in the shuttle plasmid, suggesting that the bla gene is transcribed from a promoter in that segment of DNA.

16. Document ID: JP 10248578 A

L3: Entry 16 of 28

File: JPAB

Sep 22, 1998

PUB-NO: JP410248578A
DOCUMENT-IDENTIFIER: JP 10248578 A
TITLE: EXPRESSION VECTOR FOR BACTERIUM OF GENUS
RHODOCOCCLUS

PUBN-DATE: September 22, 1998

INT-CL (IPC): C12N 15/09; C07H 21/04; C12N 1/21

APPL-NO: JP09065618
APPL-DATE: March 5, 1997

IN: MIZUMURA, YURIE, TO, FUJIO

AB: PROBLEM TO BE SOLVED: To obtain a general-purpose expression vector for a bacterium of the genus Rhodococcus, containing a mutant type regulatory factor having actions on constituent activation of a nitrilase gene promoter and capable of highly expressing the objective gene., SOLUTION: This expression vector for a bacterium of the genus Rhodococcus comprises a DNA region capable of coding a regulatory factor having actions on the activation of a nitrilase gene promoter, a nitrilase promoter gene DNA region undergoing the activation by the regulatory factor, a DNA region capable of proliferating in the bacterium of the genus Rhodococcus and a drug-resistant DNA region capable of functioning in the bacterium of the genus Rhodococcus. An exogenote is integrated into the expression vector for the bacterium of the genus Rhodococcus and made to coexist in the bacterial cell of the genus Rhodococcus to thereby enable the constituent expression of the exogenote., COPYRIGHT: (C)1998,JPO

L3: Entry 16 of 28

File: JPAB

Sep 22, 1998

DOCUMENT-IDENTIFIER: JP 10248578 A
TITLE: EXPRESSION VECTOR FOR BACTERIUM OF GENUS
RHODOCOCCLUS

FPAR:

PROBLEM TO BE SOLVED: To obtain a general-purpose expression vector for a bacterium of the genus Rhodococcus, containing a mutant type regulatory factor having actions on constituent activation of a nitrilase gene promoter and capable of highly expressing the objective gene.

FPAR:

SOLUTION: This expression vector for a bacterium of the genus Rhodococcus comprises a DNA region capable of coding a regulatory factor having actions on the activation of a nitrilase gene promoter, a nitrilase promoter gene DNA region undergoing the activation by the regulatory factor, a DNA region capable of proliferating in the bacterium of the genus Rhodococcus and a drug-resistant DNA region capable of functioning in the bacterium of the genus Rhodococcus. An exogenote is integrated into the expression vector for the bacterium of the genus Rhodococcus and made to coexist in the

bacterial cell of the genus Rhodococcus to thereby enable the constituent expression of the exogenote.

17. Document ID: JP 09028380 A

L3: Entry 17 of 28

File: JPAB

Feb 4, 1997

PUB-NO: JP409028380A
DOCUMENT-IDENTIFIER: JP 09028380 A
TITLE: CONTROL FACTOR RELATED TO EXPRESSION OF
NITRILASE GANE AND THE GANE

PUBN-DATE: February 4, 1997

INT-CL (IPC): C12N 15/09; C07H 21/04; C07K 14/195; C12N 1/21;
C12N 9/78

APPL-NO: JP07185626
APPL-DATE: July 21, 1995

IN: SHIMIZU, AKIRA, KOBAYASHI, TATSUHIKO

AB: PROBLEM TO BE SOLVED: To obtain a new control gene containing a specific amino acid sequence, having an action for activating a nitrilase gene promoter, and useful for the activation of enzyme production for producing an organic acid from the corresponding nitrile, the high expression of a different kind of protein, etc., SOLUTION: This new control factor has an amino acid sequence containing an amino acid sequence of the formula, has an action for activating a nitrilase gene promoter, can activate the production of nitrilase for producing an organic acid from the corresponding nitrile compound, and can be applied to the high expression of different kinds of proteins, etc. The control factor is obtained by treating a plasmid pNJ 10 containing a nitrilase gene originated from Rhodococcus rhodochrous J1 strain (FERM BP-1478) with a restriction enzyme to cleave out the nitrilase gene. The gene in which the part lower than the 0.5kb downstream site of the nitrile gene is defected does not express the nitrilase. It is thereby recognized that the part codes the control factor related to the expression of the nitrilase gene., COPYRIGHT: (C)1997,JPO

L3: Entry 17 of 28

File: JPAB

Feb 4, 1997

DOCUMENT-IDENTIFIER: JP 09028380 A
TITLE: CONTROL FACTOR RELATED TO EXPRESSION OF
NITRILASE GANE AND THE GANE

FPAR:

SOLUTION: This new control factor has an amino acid sequence containing an amino acid sequence of the formula, has an action for activating a nitrilase gene promoter, can activate the production of nitrilase for producing an organic acid from the corresponding nitrile compound, and can be applied

to the high expression of different kinds of proteins, etc. The control factor is obtained by treating a plasmid pNJ 10 containing a nitrilase gene originated from *Rhodococcus rhodochrous* J1 strain (FERM BP-1478) with a restriction enzyme to cleave out the nitrilase gene. The gene in which the part lower than the 0.5kb downstream site of the nitrile gene is defected does not express the nitrilase. It is thereby recognized that the part codes the control factor related to the expression of the nitrilase gene.

18. Document ID: JP 08173169 A

L3: Entry 18 of 28

File: JPAB

Jul 9, 1996

PUB-NO: JP408173169A
DOCUMENT-IDENTIFIER: JP 08173169 A
TITLE: REGULATORY FACTOR FOR MANIFESTING NITRILASE GENE AND THE GENE

PUBN-DATE: July 9, 1996

INT-CL (IPC): C12N 15/09; C12N 1/21; C12N 9/78

APPL-NO: JP06337652
APPL-DATE: December 28, 1994

IN: MIZUMURA, YURIE, TO, FUJIO

AB: PURPOSE: To obtain a regulatory factor having nitrilase gene promoter-activating action, composed of plural kinds of peptide, capable of producing nitrilase through coexistence with a nitrilase gene in *Rhodococcus* microbes, and to obtain the gene coding this factor.,
CONSTITUTION: This new regulatory factor has action to activate a nitrilase gene promoter, being composed of a polypeptide having an amino acid sequence of formula I and a second polypeptide having an amino acid sequence of formula II. The other objective new gene codes for this regulatory factor. Coexistence of this gene with a nitrilase gene containing a promoter domain in *Rhodococcus* microbes enables nitrilase production. The regulatory factor and the gene are obtained by the following process: chromosome DNAs are separated from a *Rhodococcus* erythropolis SK92 strain, a DNA library is prepared by using these DNAs, and then screened to take the gene coding for the regulatory factor, and the gene is then manifested to obtain the regulatory factor., COPYRIGHT: (C)1996,JPO

L3: Entry 18 of 28

File: JPAB

Jul 9, 1996

DOCUMENT-IDENTIFIER: JP 08173169 A
TITLE: REGULATORY FACTOR FOR MANIFESTING NITRILASE GENE AND THE GENE

FPAR:
PURPOSE: To obtain a regulatory factor having nitrilase gene

promoter-activating action, composed of plural kinds of peptide, capable of producing nitrilase through coexistence with a nitrilase gene in *Rhodococcus* microbes, and to obtain the gene coding this factor.

FPAR:

CONSTITUTION: This new regulatory factor has action to activate a nitrilase gene promoter, being composed of a polypeptide having an amino acid sequence of formula I and a second polypeptide having an amino acid sequence of formula II. The other objective new gene codes for this regulatory factor.

Coexistence of this gene with a nitrilase gene containing a promoter domain in *Rhodococcus* microbes enables nitrilase production. The regulatory factor and the gene are obtained by the following process: chromosome DNAs are separated from a *Rhodococcus* erythropolis SK92 strain, a DNA library is prepared by using these DNAs, and then screened to take the gene coding for the regulatory factor, and the gene is then manifested to obtain the regulatory factor.

19. Document ID: EP 759474 A2

L3: Entry 19 of 28

File: EPAB

Feb 26, 1997

PUB-NO: EP000759474A2
DOCUMENT-IDENTIFIER: EP 759474 A2
TITLE: A regulatory factor involved in expression of nitrilase gene, and its gene

PUBN-DATE: February 26, 1997

INT-CL (IPC): C12N 15/74; C12N 5/10; C07K 14/195; C12P 7/40
EUR-CL (EPC): C07K014/195; C12N009/78, C12N015/74

APPL-NO: EP96305253
APPL-DATE: July 17, 1996

PRIORITY-DATA: JP18562695A (July 21, 1995)

IN: SHIMIZU, SAKAYU, KOBAYASHI, MICHIIHIKO

AB: CHG DATE=19990617 STATUS=O> The invention relates to a regulatory factor substantially containing an amino acid sequence represented by SEQ ID NO:1 and having the action of activating a nitrilase gene promoter, a regulatory factor gene containing DNA coding substantially for said regulatory factor, a recombinant plasmid containing said regulatory factor gene, a nitrilase gene containing a promoter region and a DNA region capable of replicating in a microorganism belonging to the genus *Rhodococcus*, and a transformant transformed with said recombinant plasmid. According to the present invention, there are provided a regulatory factor gene containing DNA coding substantially for a regulatory factor having the action of activating a nitrilase gene promoter, a recombinant plasmid containing said regulatory factor gene, and a transformant transformed with said plasmid.

L3: Entry 19 of 28

File: EPAB

Feb 26, 1997

DOCUMENT-IDENTIFIER: EP 759474 A2
TITLE: A regulatory factor involved in expression of nitrilase gene, and its gene

FPAR:
CHG DATE=19990617 STATUS=O> The invention relates to a regulatory factor substantially containing an amino acid sequence represented by SEQ ID NO:1 and having the action of activating a nitrilase gene promoter, a regulatory factor gene containing DNA coding substantially for said regulatory factor, a recombinant plasmid containing said regulatory factor gene, a nitrilase gene containing a promoter region and a DNA region capable of replicating in a microorganism belonging to the genus *Rhodococcus*, and a transformant transformed with said recombinant plasmid. According to the present invention, there are provided a regulatory factor gene containing DNA coding substantially for a regulatory factor having the action of activating a nitrilase gene promoter, a recombinant plasmid containing said regulatory factor gene, and a transformant transformed with said plasmid.

20. Document ID: EP 719862 A2

L3: Entry 20 of 28

File: EPAB

Jul 3, 1996.

PUB-NO: EP000719862A2
DOCUMENT-IDENTIFIER: EP 719862 A2
TITLE: A regulatory factor for expression of nitrilase gene and a gene thereof

PUBN-DATE: July 3, 1996

INT-CL (IPC): C12N 15/31; C07K 14/195; C12N 15/74; C12N 1/21; C12N 9/78
EUR-CL (EPC): C12N015/74; C07K014/195; C12N009/78

APPL-NO: EP95309454
APPL-DATE: December 27, 1995

PRIORITY-DATA: JP33765294A (December 28, 1994)

IN: MIZUMURA, YURIE, YU, FUJIO

AB: CHG DATE=19990617 STATUS=O> The invention relates to a two component regulatory factor which activates a nitrilase gene promoter, comprising a polypeptide having the amino acid sequence of SEQ ID No: 1 and a polypeptide having the amino acid sequence of SEQ ID No: 2. Nitrilase can be produced by introducing the DNA coding for the regulatory factor together with a nitrilase gene containing a promoter region into a microorganism of the genus *Rhodococcus*.

L3: Entry 20 of 28

File: EPAB

Jul 3, 1996

DOCUMENT-IDENTIFIER: EP 719862 A2
TITLE: A regulatory factor for expression of nitrilase gene and a gene thereof

FPAR:
CHG DATE=19990617 STATUS=O> The invention relates to a two component regulatory factor which activates a nitrilase gene promoter, comprising a polypeptide having the amino acid sequence of SEQ ID No: 1 and a polypeptide having the amino acid sequence of SEQ ID No: 2. Nitrilase can be produced by introducing the DNA coding for the regulatory factor together with a nitrilase gene containing a promoter region into a microorganism of the genus *Rhodococcus*.

21. Document ID: EP 713914 A2

L3: Entry 21 of 28

File: EPAB

May 29, 1996

PUB-NO: EP000713914A2
DOCUMENT-IDENTIFIER: EP 713914 A2
TITLE: A regulatory gene for the expression of nitrile hydratase gene

PUBN-DATE: May 29, 1996

INT-CL (IPC): C12N 15/31; C12N 15/74; C12N 1/21; C07K 14/35; C12P 13/02
EUR-CL (EPC): C07K014/195; C12N009/78; C12N015/74

APPL-NO: EP95306960
APPL-DATE: October 2, 1995

PRIORITY-DATA: JP23926394A (October 3, 1994)

IN: SHIMIZU, SAKAYU, KOBAYASHI, MICHIIHIKO

AB: CHG DATE=19990617 STATUS=O> The invention relates to a regulatory gene coding for a polypeptide having the ability to activate a promoter for a nitrile hydratase gene, a recombinant DNA containing said regulatory gene, and a transformant transformed with said recombinant DNA. The introduction of the regulatory gene of the invention along with a nitrile hydratase gene and its promoter region permits bacteria of the genus *Rhodococcus* to produce a higher level of nitrile hydratase. Other extraneous gene can also be introduced into a region downstream of the promoter to produce other proteins in high yield.

L3: Entry 21 of 28

File: EPAB

May 29, 1996

DOCUMENT-IDENTIFIER: EP 713914 A2
TITLE: A regulatory gene for the expression of nitrile hydratase gene

FPAR:
CHG DATE=19990617 STATUS=O> The invention relates to a regulatory gene coding for a polypeptide having the ability to activate a promoter for a nitrile hydratase gene, a recombinant DNA containing said regulatory gene, and a transformant transformed with said recombinant

DNA. The introduction of the regulatory gene of the invention along with a nitrile hydratase gene and its promoter region permits bacteria of the genus *Rhodococcus* to produce a higher level of nitrile hydratase. Other extraneous gene can also be introduced into a region downstream of the promoter to produce other proteins in high yield.

22. Document ID: EP 1127943 A2

L3: Entry 22 of 28

File: DWPI

Aug 29, 2001

DERWENT-ACC-NO: 2001-551402
DERWENT-WEEK: 200162
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TITLE: Plasmid vector of *Rhodococcus* for producing proteins such as enzymes involved in the removal of organic sulfur from fossil fuels, comprises a *parA* gene, genes encoding proteins involved in replication, and a genetic marker

PRIORITY-DATA: 2000IT-MI00332 (February 24, 2000)

PATENT-FAMILY:
PUB-NO

PUB-DATE

LANGUAGE
PAGES

MAIN-IPC

EP 1127943 A2

August 29, 2001

E

024

C12N015/74

APPLICATION-DATA:
PUB-NO

APPL-DATE

APPL-NO

DESCRIPTOR

EP 1127943A2

February 19, 2001

2001EP-0200582

INT-CL (IPC): C10G 32/00; C12N 15/74

IN: DE FERRA, F, MARGARIT Y ROS, I, RODRIGUEZ, F, SERBOLISCA, L P

AB: NOVELTY - Cloning vector pSM843 (I), comprising the rep genes ORF81 and *trbA*, the gene *parA*, and at least one gene which encodes a genetic marker that confers resistance to cadmium or an antibiotic, is new., DETAILED DESCRIPTION - Cloning vector (I) pSM843 comprises:
(i) rep genes ORF81 and *trbA* (II), encoding proteins involved in replication in *Rhodococcus*;
(ii) gene *parA* (III) comprising a fully defined sequence of 2290 base pairs (bp) as given in the specification,; (iii) at least one gene (IV) encoding genetic markers selected from the cad operon, that confer resistance to cadmium or antibiotics.,
INDEPENDENT CLAIMS are also included for the following:, (1) an expression vector (V) comprising:, (i) (II), (III)

and (IV);, (ii) a constitutive promoter of *Rhodococcus* comprising a sequence of 1355 bp as given in the specification,; (iii) a multiple cloning site downstream of the promoter; and, (iv) the replication origin in *Escherichia coli* (E.coli) deposited with the number CBS 102445,; (2) a microorganism (VI) such as *Rhodococcus*, *Gordona* and *Nocardia* transformed with (I) and/or (V);, (3) a strain of *Rhodococcus* (VII) transformed with (V) deposited with the number CBS 102447,; (4) a research method (II) for promoters in microorganisms capable of integrating at random fragments of foreign DNA in its chromosome without requiring a sequence homology higher than 3 bp between the donor DNA and that of the host, comprising:, (i) transforming the microorganism directly with a gene reporter without its promoter or with a multicopy plasmid of E.coli containing the gene, linearized upstream of the gene reporter,; (ii) selecting the clones which have integrated the gene reporter in their chromosome, downstream of a promoter sequence,; (iii) digesting the chromosomal DNA of the clones selected with restriction enzymes which cut upstream and downstream the gene; and, (iv) amplifying the DNA, and sequencing the promoter upstream the gene reporter; and, (5) a constitutive promoter of *Rhodococcus* comprising a fully defined 1355 bp sequence as given in the specification., USE - (II) is useful for producing homologous or heterologous proteins of interest such as enzymes involved in the selective removal of organic sulfur from fossil fuels (SoxA, SoxB, SoxC), L-amino acids, enantiomorphs of chiral compounds and carboxylic acids in a microorganism. The proteins are preferably sox enzymes. Microorganisms such as *Rhodococcus*, *Gordona* and *Nocardia* containing the sox operon downstream to the constitutive promoter, in particular *Rhodococcus* strain SMV114 CBS 102447, transformed with (I) and/or (II) are useful for removing organic sulfur from fossil fuels (claimed)., ADVANTAGE - The expression vector has high stability in the absence of selective pressure in the transformed strains of *Rhodococcus*., DESCRIPTION OF DRAWING(S) - The figure shows the restriction map of the 7.3 kb plasmid pSM843.

L3: Entry 22 of 28

File: DWPI

Aug 29, 2001

DERWENT-ACC-NO: 2001-551402
DERWENT-WEEK: 200162
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TITLE: Plasmid vector of *Rhodococcus* for producing proteins such as enzymes involved in the removal of organic sulfur from fossil fuels, comprises a *parA* gene, genes encoding proteins involved in replication, and a genetic marker

ABTX:

(ii) a constitutive promoter of *Rhodococcus* comprising a sequence of 1355 bp as given in the specification;

ABTX:

(5) a constitutive promoter of *Rhodococcus* comprising a fully defined 1355 bp sequence as given in the specification.

ABTX:

USE - (II) is useful for producing homologous or heterologous proteins of interest such as enzymes

involved in the selective removal of organic sulfur from fossil fuels (SoxA, SoxB, SoxC), L-amino acids, enantiomorphs of chiral compounds and carboxylic acids in a microorganism. The proteins are preferably sox enzymes. Microorganisms such as Rhodococcus, Gordonia and Nocardia containing the sox operon downstream to the constitutive promoter, in particular Rhodococcus strain SMV114 CBS 102447, transformed with (I) and/or (II) are useful for removing organic sulfur from fossil fuels (claimed).

23. Document ID: EP 759474 A2, JP 09028380 A, US 5830693 A

L3: Entry 23 of 28

File: DWPI

Feb 26, 1997

DERWENT-ACC-NO: 1997-147520
DERWENT-WEEK: 199925
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TITLE: Regulatory factor that activates nitrilase gene promoter - and Rhodococcus transformants with increased nitrilase activity, for converting nitrile(s) to organic acids

PRIORITY-DATA: 1995JP-0185626 (July 21, 1995)

PATENT-FAMILY:
PUB-NO

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 759474 A2	February 26, 1997	E	013	C12N015/74
JP 09028380 A	February 4, 1997		008	C12N015/09
US 5830693 A	November 3, 1998		000	C12N009/15

APPLICATION-DATA:
PUB-NO

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
EP 759474A2	July 17, 1996	1996EP-0305253	
JP09028380A	July 21, 1995	1995JP-0185626	
US 5830693A	July 19, 1996	1996US-0683908	

INT-CL (IPC): C07H 21/04; C07K 14/195; C12N 1/21; C12N 5/10; C12N 9/15; C12N 9/78; C12N 15/09; C12N 15/31; C12N 15/74; C12P 7/40; C12N 15/09; C12R 1/01; C12N 1/21; C12R 1/01; C12N 9/78; C12R 1/01

IN: KOBAYASHI, M, SHIMIZU, S

AB: New regulatory factor capable of activating a nitrilase gene promoter and contg. a 319 amino acids sequence given in the specification, is new., USE - The regulatory factor can be used to increase nitrilase expression in Rhodococcus transformants thus increasing production of an organic acid from its corresp. nitrile., New regulatory factor capable of activating a nitrilase gene promoter and contg. a 319 amino acids sequence given in the specification, is new., USE - The regulatory factor can be used to increase nitrilase expression in Rhodococcus transformants thus increasing production of an organic acid from its corresp. nitrile.

L3: Entry 23 of 28

File: DWPI

Feb 26, 1997

DERWENT-ACC-NO: 1997-147520
DERWENT-WEEK: 199925
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: Regulatory factor that activates nitrilase gene promoter - and Rhodococcus transformants with increased nitrilase activity, for converting nitrile(s) to organic acids

24. Document ID: JP 09028382 A

L3: Entry 24 of 28

File: DWPI

Feb 4, 1997

DERWENT-ACC-NO: 1997-159092
DERWENT-WEEK: 199715
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TITLE: Microorganism producing nitrilase in absence of inducer - useful as catalyst in large scale organic acid production

PRIORITY-DATA: 1995JP-0204061 (July 19, 1995)

PATENT-FAMILY:
PUB-NO

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 09028382 A	February 4, 1997		013	C12N015/09

APPLICATION-DATA:
PUB-NO

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP09028382A	July 19, 1995	1995JP-0204061	

INT-CL (IPC): C07H 21/04; C12N 1/21; C12N 9/78; C12N 15/09; C12N 15/09; C12R 1/01; C12N 1/21; C12R 1/01; C12N 9/78; C12R 1/01

IN: No data.

AB: Microorganism producing nitrilase in a medium in the absence of an inducer, is new.

Also claimed are: (1) regulatory factor for activating a nitrilase gene promoter, consisting of the 244 and 534 residue amino acid sequences given in the specification; (2) gene encoding the regulatory factor; (3) recombinant plasmid containing the gene, which is capable of replication and multiplication in a microorganism belonging to the genus *Rhodococcus*; and (4) microorganism having the gene, or transformed with the recombinant plasmid., USE - The gene product (nitrilase) is industrially useful as a catalyst for the production of organic acids, as it is hydrolyses nitriles into their corresponding acids., ADVANTAGE - The microorganism can produce nitrilase in high yield without adding any inducer to the medium. Hence, the preparation of the medium is easy for industrial large-scale culture of the microorganism, and the disposal cost of the waste culture is reduced.

L3: Entry 24 of 28

File: DWPI

Feb 4, 1997

DERWENT-ACC-NO: 1997-159092

DERWENT-WEEK: 199715

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TITLE: Microorganism producing nitrilase in absence of inducer - useful as catalyst in large scale organic acid production

AB TX:

Microorganism producing nitrilase in a medium in the absence of an inducer, is new. Also claimed are: (1) regulatory factor for activating a nitrilase gene promoter, consisting of the 244 and 534 residue amino acid sequences given in the specification; (2) gene encoding the regulatory factor; (3) recombinant plasmid containing the gene, which is capable of replication and multiplication in a microorganism belonging to the genus *Rhodococcus*; and (4) microorganism having the gene, or transformed with the recombinant plasmid.

25. Document ID: DE 69522192 E, EP 719862 A2, JP 08173169 A, US 5602014 A, CN 1133341 A, TW 387895 A, JP 3154633 B2, EP 719862 B1

L3: Entry 25 of 28

File: DWPI

Sep 20, 2001

DERWENT-ACC-NO: 1996-302346

DERWENT-WEEK: 200163

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TITLE: *Rhodococcus erythropolis* two component nitrilase gene activator - enhances activation of nitrilase gene promoter in presence of nitrile

PRIORITY-DATA: 1994JP-0337652 (December 28, 1994)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DE 69522192 E	September 20, 2001		000	
EP 719862 A2	July 3, 1996	E	023	C12N015/31
JP 08173169 A	July 9, 1996		013	C12N015/09
US 5602014 A	February 11, 1997		013	C12N015/00
CN 1133341 A	October 16, 1996		000	C12N015/55
TW 387895 A	April 21, 2000		000	C07K013/00
JP 3154633 B2	April 9, 2001		012	C07K014/195
EP 719862 B1	August 16, 2001	E	000	C12N015/31

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
DE 69522192E	December 27, 1995	1995DE-0622192	
DE 69522192E	December 27, 1995	1995EP-0309454	
DE 69522192E		EP 719862	Based on
EP 719862A2	December 27, 1995	1995EP-0309454	
JP 08173169A	December 28, 1994	1994JP-0337652	
US 5602014A	December 22, 1995	1995US-0577184	
CN 1133341A	December 28, 1995	1995CN-0119459	
TW 387895A	January 30, 1996	1996TW-0101136	
JP 3154633B2	December 28, 1994	1994JP-0337652	
JP 3154633B2		JP 8173169	Previous Publ.
EP 719862B1			

December 27, 1995

1995EP-0309454

INT-CL (IPC): C07K 13/00; C07K 14/195; C12N 1/21; C12N 9/78; C12N 15/00; C12N 15/09; C12N 15/31; C12N 15/55; C12N 15/66; C12N 15/67; C12N 15/74; C12P 21/00; C12N 1/21; C12R 1/01; C12N 1/21; C12R 1/01; C12N 1/21; C12R 1/01; C12N 9/78; C12R 1/01; C12N 1/21; C12R 1/01; C12N 9/78; C12R 1/01

IN: MIZUMURA, Y, YU, F

AB: New regulatory factor (A) which activates a nitrilase gene promoter comprising two subunit polypeptides of 244 and 534 amino acids given in the specification is claimed. Also claimed are: (1) a DNA molecule (1) encoding (A); (2) a recombinant plasmid contg. (1), a nitrilase gene contg. a promoter region and a DNA region capable of replicating in *Rhodococcus* sp.; and (3) a *Rhodococcus* transformed with the plasmid of (2)., USE - Inclusion of (1) into a *Rhodococcus* microorganism contg. a nitrilase gene contg. its promoter allows efficient prodn. of nitrilase. Nitrilase is used commercially to produce organic acids and amides by hydration or hydrolysis of their corresp. nitriles., ADVANTAGE - Previously, recombinant nitrilase genes have been ineffective due to their lacking the necessary nitrilase gene promoter activator. Prodn. of nitrilase for use in the prodn. of organic acids by biological rather than chemical methods means the acids can be produced under mild conditions., New regulatory factor (A) which activates a nitrilase gene promoter comprising two subunit polypeptides of 244 and 534 amino acids given in the specification is claimed. Also claimed are: (1) a DNA molecule (1) encoding (A); (2) a recombinant plasmid contg. (1), a nitrilase gene contg. a promoter region and a DNA region capable of replicating in *Rhodococcus* sp.; and (3) a *Rhodococcus* transformed with the plasmid of (2)., USE - Inclusion of (1) into a *Rhodococcus* microorganism contg. a nitrilase gene contg. its promoter allows efficient prodn. of nitrilase. Nitrilase is used commercially to produce organic acids and amides by hydration or hydrolysis of their corresp. nitriles., ADVANTAGE - Previously, recombinant nitrilase genes have been ineffective due to their lacking the necessary nitrilase gene promoter activator. Prodn. of nitrilase for use in the prodn. of organic acids by biological rather than chemical methods means the acids can be produced under mild conditions., A regulatory system comprises: (a) the polypeptide having a specific sequence of 244 aminoacids (SEQ ID NO:1) and (b) the polypeptide having sequence of 534 aminoacids (SEQ ID NO:2) (sequences are given in the specification).

L3: Entry 25 of 28

File: DWPI

Sep 20, 2001

DERWENT-ACC-NO: 1996-302346
DERWENT-WEEK: 200163
COPYRIGHT 2002 DERWENT INFORMATION LTD

TITLE: *Rhodococcus erythropolis* two component nitrilase gene activator - enhances activation of nitrilase gene promoter in presence of nitrile

ABTX:

New regulatory factor (A) which activates a nitrilase gene promoter comprising two subunit

polypeptides of 244 and 534 amino acids given in the specification is claimed. Also claimed are: (1) a DNA molecule (1) encoding (A); (2) a recombinant plasmid contg. (1), a nitrilase gene contg. a promoter region and a DNA region capable of replicating in *Rhodococcus* sp.; and (3) a *Rhodococcus* transformed with the plasmid of (2).

ABTX:

USE - Inclusion of (1) into a *Rhodococcus* microorganism contg. a nitrilase gene contg. its promoter allows efficient prodn. of nitrilase. Nitrilase is used commercially to produce organic acids and amides by hydration or hydrolysis of their corresp. nitriles.

ABEQ:

New regulatory factor (A) which activates a nitrilase gene promoter comprising two subunit polypeptides of 244 and 534 amino acids given in the specification is claimed. Also claimed are: (1) a DNA molecule (1) encoding (A); (2) a recombinant plasmid contg. (1), a nitrilase gene contg. a promoter region and a DNA region capable of replicating in *Rhodococcus* sp.; and (3) a *Rhodococcus* transformed with the plasmid of (2).

ABEQ:

USE - Inclusion of (1) into a *Rhodococcus* microorganism contg. a nitrilase gene contg. its promoter allows efficient prodn. of nitrilase. Nitrilase is used commercially to produce organic acids and amides by hydration or hydrolysis of their corresp. nitriles.

26. Document ID: JP 3217409 B2, EP 483797 A, US 5202248 A, JP 05192166 A, EP 483797 B1, DE 69113390 E

L3: Entry 26 of 28

File: DWPI

Oct 9, 2001

DERWENT-ACC-NO: 1992-152474
DERWENT-WEEK: 200164
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TITLE: Cloning and prodn. of nCOI restriction endonuclease and methylase - using DNA from *Nocardia corallina* ATCC 19070

PRIORITY-DATA: 1990US-0608228 (November 2, 1990)

PATENT-FAMILY:

PUB-NO

PUB-DATE

LANGUAGE

PAGES

MAIN-IPC

JP 3217409 B2
October 9, 2001

015

C12N015/09

EP 483797 A
May 6, 1992

E

019

US 5202248 A
April 13, 1993

014

C12N009/22

JP 05192166 A
August 3, 1993

022
C12N015/55

EP 483797 B1
September 27, 1995
E

018
C12N015/54

DE 69113390 E
November 2, 1995

000
C12N015/54

APPLICATION-DATA:
PUB-NO

APPL-DATE

APPL-NO

DESCRIPTOR

JP 3217409B2
November 1, 1991
1991JP-0313555

JP 3217409B2
JP 5192166
Previous Publ.

EP 483797A
October 30, 1991
1991EP-0118511

US 5202248A
November 2, 1990
1990US-0608228

JP 05192166A
November 1, 1991
1991JP-0313555

EP 483797B1
October 30, 1991
1991EP-0118511

DE 69113390E
October 30, 1991
1991DE-0613390

DE 69113390E
October 30, 1991
1991EP-0118511

DE 69113390E
EP 483797
Based on

INT-CL (IPC): C12N 1/21; C12N 9/10; C12N 9/16; C12N 9/22; C12N 15/09; C12N 15/54; C12N 15/55; C12N 1/21; C12R 1/19; C12N 15/09; C12R 1/365

IN: VANCOTT, E M

AB: A DNA fragment comprising a nucleotide sequence encoding the NcoI restriction endonuclease produced by Nocardia corallina ATCC 19070 is new., A DNA fragment comprising a nucleotide sequence encoding the NcoI methylase produced by N. corallina ATCC 19070; recombinant vectors; host cell transformed with the vectors; a recombinant NcoI which recognises the sequence CCATGG obtd. from N. corallina ATCC 19070 and free of contaminants found in native NcoI prepn.; and prodn. of NcoI by culturing the cells transformed host are also claimed.,
USE/ADVANTAGE - NcoI restriction endonuclease and modification methylase are useful tools for characterising and rearranging DNA in the laboratory. The host strains expressing them are useful as they simplify the purificn. of the enzymes and provide a means for producing the enzymes in commercially useful amts. The enzyme cleaves between the two C residues, leaving a 4 base 5' overhang, An expression vector which expresses recombinant NcoI endonuclease comprising a DNA sequence coding for NcoI restriction endonuclease, wherein said DNA sequence is obtainable from N. corallina ATCC No. 19070, and wherein said

expression vector is obtainable by replacing the endogenous regulatory sequences of the endonuclease gene found immediately downstream of the NcoI modification enzyme encoding DNA by an exogenous promoter and ribosomal binding site., Isolated DNA encoding NcoI restriction endonuclease, isolatable from vector PEV190R612-22C-29., Also claimed are: a recombinant DNA vector comprising a vector contg. a DNA sequence encoding NcoI produced by Nocardia corallina ATCC 19070; DNA encoding NcoI restriction endonuclease and methylase obtainable from ATCC Accession No. 68457; a cloning vector comprising the DNA; or has x cell transformed by the vector; and a method of producing NcoI.,
USE/ADVANTAGE - NcoI is prepd. free of contaminants normally found in conventional endonuclease prepn

L3: Entry 26 of 28

File: DWPI

Oct 9, 2001

DERWENT-ACC-NO: 1992-152474
DERWENT-WEEK: 200164
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TITLE: Cloning and prodn. of nCOI restriction endonuclease and methylase - using DNA from nocardia corallina ATCC 19070

ABEQ:

An expression vector which expresses recombinant NcoI endonuclease comprising a DNA sequence coding for NcoI restriction endonuclease, wherein said DNA sequence is obtainable from N. corallina ATCC No. 19070, and wherein said expression vector is obtainable by replacing the endogenous regulatory sequences of the endonuclease gene found immediately downstream of the NcoI modification enzyme encoding DNA by an exogenous promoter and ribosomal binding site.

27. Document ID: US 4368265 A

L3: Entry 27 of 28

File: DWPI

Jan 11, 1983

DERWENT-ACC-NO: 1983-12080K
DERWENT-WEEK: 198305
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TITLE: Antibiotic x-14868A prodn. by cultivating Nocardia sp. - useful as antibacterial, coccidiostat, ruminant growth promoter etc.

PRIORITY-DATA: 1981US-0237544 (February 28, 1981), 1980US-0116696 (January 30, 1980)

PATENT-FAMILY:

PUB-NO

PUB-DATE

LANGUAGE

PAGES

MAIN-IPC

US 4368265 A

January 11, 1983

014

INT-CL (IPC): C12P 17/18; C12P 19/60

IN: LIU, C M, PROSSER, B, WESTLEY, J

AB: Prodn. of Antibiotic X-14868A comprises cultivation of a strain of Nocardia X-14868 (i.e. ATCC 31585) in an aq. medium contg. carbohydrate and nitrogenous nutrient, under submerged aerobic conditions. The Antibiotic is then isolated from the medium., Antibiotic X-14868A is effective against Gram-positive bacteria and it is a coccidiostat in poultry and a growth promotant in ruminants, and it is active against Treponema hyodysenteriae, the etiologic agent of swine dysentery. This is described in the parent patent (56870 D/31).

L3: Entry 27 of 28

File: DWPI

Jan 11, 1983

DERWENT-ACC-NO: 1983-12080K

DERWENT-WEEK: 198305

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TITLE: Antibiotic x-14868A prodn. by cultivating Nocardia sp. - useful as antibacterial, coccidiostat, ruminant growth promoter etc.

28. Document ID: US 4278663 A, CA 1160972 A, DE 3160616 G, DK 8100298 A, DK 8600250 A, EP 35119 A, EP 35119 B, FI 8100252 A, HU 27166 T, IL 61971 A, JP 56120696 A, JP 90050918 B, NO 8100319 A, NO 8700452 A, PT 72417 A, ZA 8100151 A

L3: Entry 28 of 28

File: DWPI

Jul 14, 1981

DERWENT-ACC-NO: 1981-56870D

DERWENT-WEEK: 198131

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TITLE: Antibiotics X-14868 A, B, C and D from Nocardia sp. ATCC 31585 - useful as antibacterials, anticoccidials and ruminant growth promoters

PRIORITY-DATA: 1981US-0237544 (February 28, 1981), 1980US-0116696 (January 30, 1980)

PATENT-FAMILY:

PUB-NO

PUB-DATE

LANGUAGE

PAGES

MAIN-IPC

US 4278663 A

July 14, 1981

014

CA 1160972 A

January 24, 1984

000

DE 3160616 G

August 25, 1983

000

DK 8100298 A

October 5, 1981

DK 8600250 A 000

January 17, 1986

000

EP 35119 A

September 9, 1981

G

000

EP 35119 B

July 20, 1983

G

000

FI 8100252 A

September 30, 1981

000

HU 27166 T

October 28, 1983

000

IL 61971 A

January 31, 1984

000

JP 56120696 A

September 22, 1981

000

JP 90050918 B

November 5, 1990

000

NO 8100319 A

August 24, 1981

000

NO 8700452 A

April 13, 1987

000

PT 72417 A

April 6, 1982

000

ZA 8100151 A

December 15, 1981

000

APPLICATION-DATA:

PUB-NO

APPL-DATE

APPL-NO

DESCRIPTOR

JP90050918B

January 29, 1981

1981JP-0012193

INT-CL (IPC): A23K 1/16; A61K 31/71; A61K 35/74; C07D 493/10; C07G 11/00; C07H 17/04; C08G 0/00; C12P 17/18; C12P 19/60 ; C12R 1/36

IN: LIU, C M, PROSSER, B, WESTLEY, J

AB: Antibiotics X-14868A-D of formula (I) (A-B) and their salts are new, (either (1) X1 is Me, X2 is ---Me, X3 is absent and R is H, or (2) X1 is absent, X2 is ---Me, X3 is Me and R is Me, respectively). The X-14868C melts at 172-5 deg.C (Na salt), gives a microanalysis of 57.12%C, 8.58%H and 2.36%Na and has characterising i.r. spectrum and specific rotation. The X-14868D melts at 194-5 deg. C (Na salt) gives a microanalysis of 60.32%C, 8.80%H and 3.40%Na and has characterising i.r. spectrum and specific rotation. The antibiotics are active against certain Gram-positive bacteria and so are sterilising agents and they show anticoccidiostatic activity. X-14868A improves ruminant feed utilisation and prevents and treats ketosis, and it is active against Treponema hyodysenteriae, the etiologic agent of swine dysentery.

L3: Entry 28 of 28

File: DWPI

Jul 14, 1981

DERWENT-ACC-NO: 1981-56870D

DERWENT-WEEK: 198131

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TITLE: Antibiotics X-14868 A, B, C and D from Nocardia sp. ATCC
31585 - useful as antibacterials,
anticoccidials and ruminant growth promoters

09/446681
Att #14

=> s rhodococcus or nocardia or corallina
L1 25380 RHODOCOCCUS OR NOCARDIA OR CORALLINA

=> s promoter?
L2 412671 PROMOTER?

=> s l1 and l2
L3 276 L1 AND L2

=> dup rem l3
PROCESSING COMPLETED FOR L3
L4 127 DUP REM L3 (149 DUPLICATES REMOVED)

=> s l4 and py<1998

1 FILES SEARCHED...
3 FILES SEARCHED...
4 FILES SEARCHED...
L5 84 L4 AND PY<1998

=> d l5 ibib abs 1-84

L5 ANSWER 1 OF 84 BIOSIS COPYRIGHT 2002 BIOLOGICAL
ABSTRACTS INC.
ACCESSION NUMBER: 1997:414292 BIOSIS
DOCUMENT NUMBER: PREV199799706335
TITLE: Amy as a reporter gene for ***promoter*** activity in
Nocardia lactamdurans: Comparison of
promoters of the cephamycin cluster.
AUTHOR(S): Chary, Vasant K.; De La Fuente, Juan L.; Liras, Paloma;
Martin, Juan F. (1)
CORPORATE SOURCE: (1) Area Microbiol., Fac. Biol., Univ. Leon,
24071 Leon
Spain
SOURCE: Applied and Environmental Microbiology, (1997) Vol. 63,
No.

8, pp. 2977-2982.
ISSN: 0099-2240.

DOCUMENT TYPE: Article
LANGUAGE: English

AB ***Promoter*** probe vectors containing the pA origin of replication
and the Streptomyces griseus ***promoterless*** amy gene (encoding
alpha-amylase) as reporter have been constructed to study transcription
initiation regions in ***Nocardia*** lactamdurans. In some of the
promoter probe vectors the phage fd terminator has been
introduced
to avoid readthrough expression from upstream sequences. By using these
vectors, four different transcription initiation regions of the cephamycin
gene cluster have been studied in N. lactamdurans. The bla gene encoding

a
beta-lactamase has a relatively strong ***promoter***. Two other
separate ***promoters*** corresponding to the lat and cefD genes
(encoding, respectively, lysine-6-aminotransferase and isopenicillin
N-epimerase) showed weak transcription initiation ability. These two
promoters are arranged in a bidirectional transcription initiation
region located in the center of the cephamycin gene cluster. The cmcH
gene
(encoding 3-hydroxymethylcephem carbamoyltransferase) upstream region
did
not contain a functional ***promoter***, suggesting that cmcH is
transcribed as a part of a polycistronic mRNA. The native amy
promoter is used very efficiently in N. lactamdurans, resulting in
secretion of high levels of extracellular alpha-amylase.

L5 ANSWER 2 OF 84 BIOSIS COPYRIGHT 2002 BIOLOGICAL
ABSTRACTS INC.
ACCESSION NUMBER: 1997:273176 BIOSIS
DOCUMENT NUMBER: PREV199799564894
TITLE: Genes encoding the NAD-reducing hydrogenase of
Rhodococcus opacus MR11.
AUTHOR(S): Grzeszik, Claudia; Luebbbers, Meike; Reh, Michael;
Schlegel,
Hans G. (1)
CORPORATE SOURCE: (1) Institut fuer Mikrobiologie,
Georg-August-Universitaet
Goettingen, Grisebachstrasse 8, D-37077 Goettingen Germany
SOURCE: Microbiology (Reading), (1997) Vol. 143, No. 4, pp.
1271-1286.

ISSN: 1350-0872.

DOCUMENT TYPE: Article
LANGUAGE: English

AB The dissociation of the soluble NAD-reducing hydrogenase of
Rhodococcus opacus MR11 into two dimeric proteins with
different

catalytic activities and cofactor composition is unique among the
NAD-reducing hydrogenases studied so far. The genes of the soluble
hydrogenase were localized on a 7.4 kbp AsnI fragment of the linear
plasmid pHG201 via heterologous hybridization. Analysis of the
nucleotide
sequence of this fragment revealed the seven open reading frames ORF1,
hoxF, -U, -Y, -H, -W and ORF7. The six latter ORFs belong to the gene
cluster of the soluble hydrogenase. Their gene products are highly
homologous to those of the NAD-reducing enzyme of Alcaligenes
eutrophus

H16. The genes hoxF, -U, -Y and -H encode the subunits alpha, gamma,
delta

and beta, respectively. The gene hoxW encodes a putative protease, which
may be essential for C-terminal processing of the beta subunit. Finally,
ORF7 encodes a protein which has similarities to cAMP- and
cGMP-binding

protein kinases, but its function is not known. ORF1, which lies upstream
of the hydrogenase gene cluster, encodes a putative transposase found in
IS elements of other bacteria. Northern hybridizations and primer
extensions using total RNA of autotrophically and heterotrophically grown
cells of R. opacus MR11 indicated that the hydrogenase genes are under
control of sigma-70-like ***promoter*** located at the right end of
ORF1 and are even transcribed under heterotrophic conditions at a low
level. Furthermore, this ***promoter*** was shown to be active in the
recombinant Escherichia coli strain LHY1 harbouring the 7.4 kbp AsnI
fragment, resulting in overexpression of the hydrogenase genes. Although
all four subunits of the soluble hydrogenase were shown via Western
immunoblots to be synthesized in E. coli, no active enzyme was
detectable.

L5 ANSWER 3 OF 84 BIOSIS COPYRIGHT 2002 BIOLOGICAL
ABSTRACTS INC.
ACCESSION NUMBER: 1997:248656 BIOSIS
DOCUMENT NUMBER: PREV199799547859
TITLE: The expediency of using the sludge biomass as a microbial
fertilizer.
AUTHOR(S): Arkhipchenko, I. A.
CORPORATE SOURCE: All-Russ. Res. Inst. Agric. Microbiol., St.
Petersburg-Pushkin 189620 Russia
SOURCE: Doklady Rossiiskoi Akademii Sel'skokhozyaistvennykh
Nauk,
(1997) Vol. 0, No. 1, pp. 35-37.
ISSN: 1068-3674.

DOCUMENT TYPE: Article
LANGUAGE: Russian

SUMMARY LANGUAGE: Russian; English

AB The microflora and microfauna in the pigfarm aerotanks containing the
activated sludges were investigated. It was shown that a principally new
type of biocenosis is formed in the pigfarm waste water which is basically
presented by nocardio- and coryne-like bacteria (genera:
Rhodococcus, Arthrobacter, Mycobacterium and others),
gram-negative rods (genera: Alcaligenes, Pseudomonas) and appendages
bacteria (genus Caulobacter). The microfauna is presented by flagellates
of Bodo and Monas genera and the infusoria of Opercularia, Vorticella,
Epistylis genera. The constant content of the microflora and microfauna in
the biocenosis of the activated sludge and the prevalence of nocardioforms
and corynebacteria which contain many growth ***promoters***
confirms

the expediency of using the sludge biomass as a microbial fertilizer.

L5 ANSWER 4 OF 84 BIOSIS COPYRIGHT 2002 BIOLOGICAL
ABSTRACTS INC.
ACCESSION NUMBER: 1997:179787 BIOSIS
DOCUMENT NUMBER: PREV199799471500
TITLE: Heterologous expression of biphenyl dioxygenase-encoding
genes from a gram-positive broad-spectrum polychlorinated
biphenyl degrader and characterization of chlorobiphenyl
oxidation by the gene products.
AUTHOR(S): McKay, David B.; Seeger, Michael; Zielinski, Marco;
Hofer,
Bernd (1); Timmis, Kenneth N.
CORPORATE SOURCE: (1) Dep. Microbiol., Gesellschaft fuer

09/446681
A#21

=> s corallina
L1 2598 CORALLINA

=> s promoter?
L2 464493 PROMOTER?

=> s I1 and I2

L3 7 L1 AND L2

=> dup rem I3
PROCESSING COMPLETED FOR L3
L4 4 DUP REM L3 (3 DUPLICATES REMOVED)

=> d I4 ibib abs 1-4

L4 ANSWER 1 OF 4 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 2000:457212 HCAPLUS
DOCUMENT NUMBER: 133:85125
TITLE: A chemically inducible expression system for
eukaryotes using the OHP system of Rhodococcus
INVENTOR(S): Turk, Jutta Anna; Archer, John Anthony Charles
PATENT ASSIGNEE(S): Advanced Technologies (Cambridge) Ltd., UK
SOURCE: PCT Int. Appl., 117 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000039300	AI	20000706	WO 1999-GB4333	19991221
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2356125	AA	20000706	CA 1999-2356125	19991221
EP 1141307	A1	20011010	EP 1999-962383	19991221
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
PRIORITY APPLN. INFO.: GB 1998-28660 A 19981224 WO 1999-GB4333 W 19991221				

AB A chem. inducible gene expression system for eukaryotes, esp. plants, that uses elements of the o-hydroxyphenylpropionic acid (OHP) utilization operon of Rhodococcus ***corallina*** (Nocardia ***corallina***) is described. The system uses a ***promoter*** regulated by the OHP-responsive transcriptional activator ohpR to activate expression through its cognate C1 element. The ohpR gene is expressed from a host ***promoter***, which may be constitutive or regulated. Various domain regions and complementary response elements are also described.
REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L4 ANSWER 2 OF 4 HCAPLUS COPYRIGHT 2003 ACS
ACCESSION NUMBER: 1999:42607 HCAPLUS
DOCUMENT NUMBER: 130:91274
TITLE: Engineering mycolic acid bacterial biosensors with
promoters induced by specific analytes, and
use for detection of pollutants
INVENTOR(S): Archer, John Anthony Charles; Summers, David

Keith;

Roland, Herve Jacquiau; Powell, Justin Antoine
Christian

PATENT ASSIGNEE(S): Cambridge University Technical Services
Limited, UK

SOURCE: PCT Int. Appl., 67 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9900517	A2	19990107	WO 1998-GB1893	19980629
WO 9900517	A3	19990514		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9882253	A1	19990119	AU 1998-82253	19980629
EP 991778	A1	20000412	EP 1998-932307	19980629
R: DE, DK, ES, FR, GB, IT, NL, SE, PT				
PRIORITY APPLN. INFO.: GB 1997-13666 19970627 WO 1998-GB1893 19980629				

AB Disclosed are methods for generating mycolic acid bacterial biosensors for

particular analytes (esp. industrial pollutants) by isolating DNA encoding an inducible ***promoter*** which induced in response to the analyte (and/or assocd. operon proteins). The method generally comprises the steps of: (a) culturing a source of mycolic acid bacteria in a selective medium contg. said specific analyte and being selective for oligotrophic bacteria; (b) identifying mycolic acid bacteria capable of subsisting on said medium, esp. those which do not display catabolic repression; (c) extg. DNA from said mycolic acid bacteria; (d) incorporating said DNA into vectors, such as various shuttle vectors; (e) cloning said vector into a suitable host cell (which may be E. coli strain carrying one or more of the mcrABC nmr hsdSRM recA and recO mutations); (f) screening that host cell (or a second host cell which is preferably a corynebacterium) for said inducible ***promoter***. The methods are exemplified by the isolation of the R. ***corallina*** ohp operon. Also disclosed are assocd. materials, e.g. media, vectors, nucleic acid probes for performing the invention, and biosensors produced by the methods of the invention plus methods of use of the same.

L4 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL
ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 1995:77378 BIOSIS
DOCUMENT NUMBER: PREV199598091678
TITLE: Cloning and Characterization of a Nocardia
corallina B-276 gene Cluster Encoding Alkene
Monooxygenase.
AUTHOR(S): Saeki, Hisashi (1); Furuhashi, Keizo
CORPORATE SOURCE: (1) Pharmaceuticals Biotechnol. Lab., Japan
Energy Co.,
3-17-35 Niizo-Minami, Toda, Saitama 335 Japan
SOURCE: Journal of Fermentation and Bioengineering, (1994) Vol.
78,
No. 6, pp. 399-406.
ISSN: 0922-338X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Alkene monooxygenase (AMO) of Nocardia ***corallina*** B-276,
which consists of three components, epoxidase, reductase and coupling protein,
catalyses the oxidation of alkenes to the corresponding epoxides. The

NH-2

terminal amino acid sequences of the large and small subunits of the epoxidase were used for the preparation of synthetic oligonucleotides as hybridization probes. A 6.4-kb BamHI fragment, which contained DNA sequences hybridizing to the probes, was cloned in Escherichia coli. DNA sequencing and comparison of the determined NH-2 terminal amino acid sequences identified a four-gene cluster, amoABCD, within this region.

The

subunits of epoxidase (small and large), reductase and coupling protein were encoded by amoA, amoC, amoD and amoB, respectively. When the cloned

amoABCD gene cluster was placed under the control of a lac

promoter on a recombinant pUC18 plasmid in E. coli and induced

with isopropyl beta-D-thiogalactopyranoside, epoxidation activities were expressed. The amoABCD genes show a homology to the reported nucleotide

sequence for methane monooxygenase from methanotrophic bacteria. The existence of a conserved pair of the amino acid sequence Glu-X-X-His in the large subunit of epoxidase is consistent with the assignment of the epoxidase to the class of O-2-activating proteins containing diiron-oxo clusters.

L4 ANSWER 4 OF 4 WPIDS (C) 2003 THOMSON DERWENT
DUPLICATE 2

ACCESSION NUMBER: 1992-152474 [19] WPIDS

TITLE: Cloning and prodn. of nCOI restriction endonuclease and methylase - using DNA from nocardia ***corallina***
ATCC 19070.

DERWENT CLASS: B04 D16

INVENTOR(S): VANCOTT, E M

PATENT ASSIGNEE(S): (NEWE) NEW ENGLAND BIOLABS INC

COUNTRY COUNT: 5
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 483797	A	19920506 (199219)*	EN	19	
	R:	DE FR GB			
US 5202248	A	19930413 (199317)	14		
JP 05192166	A	19930803 (199335)	22		
EP 483797	B1	19950927 (199543)	EN	18	
	R:	DE FR GB			
DE 69113390	E	19951102 (199549)			
JP 3217409	B2	20011009 (200164)	15		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 483797	A	EP 1991-118511	19911030
US 5202248	A	US 1990-608228	19901102
JP 05192166	A	JP 1991-313555	19911101
EP 483797	B1	EP 1991-118511	19911030
DE 69113390	E	DE 1991-613390	19911030
		EP 1991-118511	19911030
JP 3217409	B2	JP 1991-313555	19911101

FILING DETAILS:

PATENT NO	KIND	PATENT NO
DE 69113390	E Based on	EP 483797
JP 3217409	B2 Previous Publ.	JP 05192166

PRIORITY APPLN. INFO: US 1990-608228 19901102

AN 1992-152474 [19] WPIDS

AB EP 483797 A UPAB: 19931006

A DNA fragment comprising a nucleotide sequence encoding the NcoI restriction endonuclease produced by Nocardia ***corallina*** ATCC 19070 is new.

A DNA fragment comprising a nucleotide sequence encoding the NcoI methylase produced by N ***corallina*** ATCC 19070; recombinant vectors; host cell transformed with the vectors; a recombinant NcoI which recognises the sequence CCATGG obtd. from N, ***corallina***

ATCC

19070 and free of contaminants found in native NcoI preps.; and prodn. of

NcoI by culturing the cells transformed host are also claimed.

USE/ADVANTAGE - NcoI restriction endonuclease and modification methylase are useful tools for characterising and rearranging DNA in the laboratory. The host strains expressing them are useful as they simplify the purificn. of the enzymes and provide a means for producing the enzymes

in commercially useful amts. The enzyme cleaves between the two C residues, leaving a 4 base 5' overhang
0/4

ABEQ US 5202248 A UPAB: 19931025

Isolated DNA encoding NcoI restriction endonuclease, isolatable from vector PEV19OR612-22C-29.

Also claimed are: a recombinant DNA vector comprising a vector contg.

a DNA sequence encoding NcoI produced by Nocardia ***corallina***

ATCC

19070; DNA encoding NcoI restriction endonuclease and methylase obtainable

from ATCC Accession No. 68457; a cloning vector comprising the DNA; or has

x cell transformed by the vector; and a method of producing NcoI.

USE/ADVANTAGE - NcoI is prepd. free of contaminants normally found in

conventional endonuclease preps.

Dwg.0/5

ABEQ EP 483797 B UPAB: 19951102

An expression vector which expresses recombinant NcoI endonuclease comprising a DNA sequence coding for NcoI restriction endonuclease, wherein said DNA sequence is obtainable from N. ***corallina***

ATCC

No. 19070, and wherein said expression vector is obtainable by replacing the endogenous regulatory sequences of the endonuclease gene found immediately downstream of the NcoI modification enzyme encoding DNA by an

exogenous ***promoter*** and ribosomal binding site.

Dwg.0/4

=> d his

(FILE 'HOME' ENTERED AT 15:53:23 ON 08 APR 2003)

FILE 'BIOSIS, EMBASE, MEDLINE, WPIDS, HCAPLUS' ENTERED AT 15:53:33 ON 08

APR 2003

L1 2598 S CORALLINA

L2 464493 S PROMOTER?

L3 7 S L1 AND L2

L4 4 DUP REM L3 (3 DUPLICATES REMOVED)

=> s ohp or mac

L5 37885 OHP OR MAC

=> s l1 and l5

L6 2 L1 AND L5

=> dup rem l6

PROCESSING COMPLETED FOR L6

L7 2 DUP REM L6 (0 DUPLICATES REMOVED)

=> d l7 ibib abs 1-2

L7 ANSWER 1 OF 2 HCAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 2000:457212 HCAPLUS

DOCUMENT NUMBER: 133:85125

TITLE: A chemically inducible expression system for eukaryotes using the ***OHP*** system of Rhodococcus

INVENTOR(S): Turck, Jutta Anna; Archer, John Anthony Charles

PATENT ASSIGNEE(S): Advanced Technologies (Cambridge) Ltd., UK

SOURCE: PCT Int. Appl., 117 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE
 WO 2000039300 A1 20000706 WO 1999-GB4333 19991221
 W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
 RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
 CA 2356125 AA 20000706 CA 1999-2356125 19991221
 EP 1141307 A1 20011010 EP 1999-962383 19991221
 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO
 PRIORITY APPLN. INFO.: GB 1998-28660 A 19981224
 WO 1999-GB4333 W 19991221

AB A chem. inducible gene expression system for eukaryotes, esp. plants, that uses elements of the o-hydroxyphenylpropionic acid (***OHP***) utilization operon of Rhodococcus ***corallina*** (Nocardia ***corallina***) is described. The system uses a promoter regulated by the ***OHP*** -responsive transcriptional activator ohpR to activate expression through its cognate C1 element. The ohpR gene is expressed from a host promoter, which may be constitutive or regulated. Various domain regions and complementary response elements are also described.
 REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES
 AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE
 FORMAT

L7 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2003 ACS
 ACCESSION NUMBER: 1999:42607 HCAPLUS
 DOCUMENT NUMBER: 130:91274
 TITLE: Engineering mycolic acid bacterial biosensors with promoters induced by specific analytes, and use for detection of pollutants
 INVENTOR(S): Archer, John Anthony Charles; Summers, David Keith; Roland, Herve Jacquiau; Powell, Justin Antoine Christian
 PATENT ASSIGNEE(S): Cambridge University Technical Services Limited, UK
 SOURCE: PCT Int. Appl., 67 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE
 WO 9900517 A2 19990107 WO 1998-GB1893 19980629
 WO 9900517 A3 19990514
 W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
 RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG
 AU 9882253 A1 19990119 AU 1998-82253 19980629
 EP 991778 A1 20000412 EP 1998-932307 19980629

R: DE, DK, ES, FR, GB, IT, NL, SE, PT
 PRIORITY APPLN. INFO.: GB 1997-13666 19970627
 WO 1998-GB1893 19980629
 AB Disclosed are methods for generating mycolic acid bacterial biosensors for particular analytes (esp. industrial pollutants) by isolating DNA encoding an inducible promoter which induced in response to the analyte (and/or assocd. operon proteins). The method generally comprises the steps of: (a) culturing a source of mycolic acid bacteria in a selective medium contg. said specific analyte and being selective for oligotrophic bacteria; (b) identifying mycolic acid bacteria capable of subsisting on said medium, esp. those which do not display catabolic repression; (c) extg. DNA from said mycolic acid bacteria; (d) incorporating said DNA into vectors, such as various shuttle vectors; (e) cloning said vector into a suitable host cell (which may be E. coli strain carrying one or more of the mcrABC nrr hsdSRM recA and recO mutations); (f) screening that host cell (or a second host cell which is preferably a corynebacterium) for said inducible promoter. The methods are exemplified by the isolation of the R. ***corallina*** ***ohp*** operon. Also disclosed are assocd. materials, e.g. media, vectors, nucleic acid probes for performing the invention, and biosensors produced by the methods of the invention plus methods of use of the same.

=> s operon or orf
 L8 92098 OPERON OR ORF
 => s l1 and l8\
 L9 0 L1 AND L8\
 => s l1 and l8
 L10 11 L1 AND L8
 => dup rem l10
 PROCESSING COMPLETED FOR L10
 L11 7 DUP REM L10 (4 DUPLICATES REMOVED)
 => d l11 ibib abs 1-7

L11 ANSWER 1 OF 7 HCAPLUS COPYRIGHT 2003 ACS
 ACCESSION NUMBER: 2002:394291 HCAPLUS
 DOCUMENT NUMBER: 137:275556
 TITLE: Phylogenetic relationships among streptophytes as inferred from chloroplast small and large subunit rRNA gene sequences
 AUTHOR(S): Turnel, Monique; Ehara, Megumi; Otis, Christian; Lemieux, Claude
 CORPORATE SOURCE: Canadian Institute for Advanced Research, Department de Biochimie et de Microbiologie, Universite Laval, Quebec, QC, G1K 7P4, Can.
 SOURCE: Journal of Phycology (2002), 38(2), 364-375
 CODEN: JPYLAJ; ISSN: 0022-3646
 PUBLISHER: Blackwell Publishing, Inc.
 DOCUMENT TYPE: Journal
 LANGUAGE: English
 AB To gain insights into the phylogeny of charophytes and into their relationships with other green algae and bryophytes, we analyzed the chloroplast small and large subunit rRNA sequences of charophytes belonging to five orders (Charales, Coleochaetales, Desmidiaceae, Klebsormidiales, and Zygnematales), of charophytes from the four remaining classes of green algae, and of bryophytes representing the three classes reported in this group of land plants. We also probed the gene organization and intron content of the chloroplast rDNA ***operon*** in charophytes and bryophytes. The organization of this ***operon*** proved to be highly conserved, except in members of the Desmidiaceae and Zygnematales. Homologous group II introns were identified in the trnA(UGC) gene of all charophyte groups examd. and in the trnI(GAU) gene of charophytes from all orders except the Desmidiaceae and Zygnematales. Phylogenetic analyses of concatenated rDNA sequences consistently placed the prasinophyte Mesostigma viride Lauterborn at the base of the Streptophyta and Chlorophyta, although alternative topologies positioning Mesostigma within the Streptophyta could not be rejected. A sister group relationship was unambiguously established between Chaetosphaeridium